

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
28 August 2003 (28.08.2003)

PCT

(10) International Publication Number  
**WO 03/070972 A2**

- (51) International Patent Classification<sup>7</sup>: C12Q [US/US]; 576 Manorwood Lane, Louisville, CO 80027 (US).
- (21) International Application Number: PCT/US03/05234
- (22) International Filing Date: 20 February 2003 (20.02.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
- |            |                               |    |
|------------|-------------------------------|----|
| 60/358,580 | 20 February 2002 (20.02.2002) | US |
| 60/363,124 | 11 March 2002 (11.03.2002)    | US |
| 60/386,782 | 6 June 2002 (06.06.2002)      | US |
| 60/404,039 | 15 August 2002 (15.08.2002)   | US |
| 60/406,784 | 29 August 2002 (29.08.2002)   | US |
| 60/408,378 | 5 September 2002 (05.09.2002) | US |
| 60/409,293 | 9 September 2002 (09.09.2002) | US |
| 60/439,922 | 14 January 2003 (14.01.2003)  | US |
| 60/440,129 | 15 January 2003 (15.01.2003)  | US |
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- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:  
— without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 03/070972 A2

(54) Title: RNA INTERFERENCE MEDIATED INHIBITION OF CHROMOSOME TRANSLOCATION GENE EXPRESSION USING SHORT INTERFERING NUCLEIC ACID (siNA)

(57) Abstract: The present invention concerns methods and reagents useful in modulating modulating chromosomal translocation gene expression gene expression in a variety of applications, including use in therapeutic, diagnostic, target validation, and genomic discovery applications. Specifically, the invention relates to small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against modulating chromosomal translocation gene expression and/or activity. The small nucleic acid molecules are useful in the diagnosis and treatment of cancer, proliferative diseases, and any other disease or condition that responds to modulation of BCR-ABL, TEL-AML1, EWS-FLI1, TLS-FUS, PAX3-FKHR, and/or AML1-ETO fusion gene expression or activity.

**RNA INTERFERENCE MEDIATED INHIBITION OF CHROMOSOME  
TRANSLOCATION GENE EXPRESSION USING SHORT INTERFERING  
NUCLEIC ACID (siNA)**

This invention claims the benefit of McSwiggen, USSN 60/404,039, filed August 15,  
5 2002; McSwiggen, USSN 60/439,922 filed January 14, 2003, of Beigelman USSN  
60/358,580 filed February 20, 2002, of Beigelman USSN 60/363,124 filed March 11, 2002,  
of Beigelman USSN 60/386,782 filed June 6, 2002, of Beigelman USSN 60/406,784 filed  
August 29, 2002, of Beigelman USSN 60/408,378 filed September 5, 2002, of Beigelman  
USSN 60/409,293 filed September 9, 2002, and of Beigelman USSN 60/440,129 filed  
10 January 15, 2003. These applications are hereby incorporated by reference herein in their  
entireties, including the drawings.

Field Of The Invention

The present invention concerns compounds, compositions, and methods for the study,  
diagnosis, and treatment of conditions and diseases that respond to the modulation of fusion  
15 gene expression and/or activity. The present invention also concerns compounds,  
compositions, and methods relating to conditions and diseases that respond to the modulation  
of expression and/or activity of genes involved in fusion gene (e.g., BCR-ABL, and EWS-  
ERG) pathways. Specifically, the invention relates to small nucleic acid molecules, such as  
short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA  
20 (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of  
mediating RNA interference (RNAi) against fusion gene expression, such as BCR-ABL and  
EWS-ERG expression.

Background Of The Invention

The following is a discussion of relevant art pertaining to RNAi. The discussion is  
25 provided only for understanding of the invention that follows. The summary is not an  
admission that any of the work described below is prior art to the claimed invention.

RNA interference refers to the process of sequence-specific post-transcriptional gene  
silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.*, 1998, *Nature*,

391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is  
5 commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The  
10 presence of dsRNA in cells triggers the RNAi response through a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III  
15 enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). Dicer has also been implicated in the excision of 21- and 22-  
20 nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target  
25 RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188).

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond *et al.*, 2000, *Nature*,

404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature*, 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877) has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21-nucleotide siRNA duplexes are most active when containing 3'-terminal dinucleotide overhangs. Furthermore, complete substitution of one or both siRNA strands with 2'-deoxy (2'-H) or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of the 3'-terminal siRNA overhang nucleotides with 2'-deoxy nucleotides (2'-H) was shown to be tolerated. Single mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end of the guide sequence (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell*, 107, 309).

Studies have shown that replacing the 3'-terminal nucleotide overhanging segments of a 21-mer siRNA duplex having two -nucleotide 3'-overhangs with deoxyribonucleotides does not have an adverse effect on RNAi activity. Replacing up to four nucleotides on each end of the siRNA with deoxyribonucleotides has been reported to be well tolerated, whereas complete substitution with deoxyribonucleotides results in no RNAi activity (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). In addition, Elbashir *et al.*, *supra*, also report that substitution of siRNA with 2'-O-methyl nucleotides completely abolishes RNAi activity. Li *et al.*, International PCT Publication No. WO 00/44914, and Beach *et al.*, International PCT Publication No. WO 01/68836 preliminarily suggest that siRNA may include modifications to either the phosphate-sugar backbone or the nucleoside to include at least one of a nitrogen or sulfur heteroatom, however, neither application postulates to what extent such modifications would be tolerated in siRNA molecules, nor provides any further guidance or examples of such modified siRNA. Kreutzer *et al.*, Canadian Patent Application No.



2,359,180, also describe certain chemical modifications for use in dsRNA constructs in order to counteract activation of double-stranded RNA-dependent protein kinase PKR, specifically 2'-amino or 2'-O-methyl nucleotides, and nucleotides containing a 2'-O or 4'-C methylene bridge. However, Kreutzer *et al.* similarly fails to provide examples or guidance as to what  
5 extent these modifications would be tolerated in siRNA molecules.

Parrish *et al.*, 2000, *Molecular Cell*, 6, 1977-1087, tested certain chemical modifications targeting the unc-22 gene in *C. elegans* using long (>25 nt) siRNA transcripts. The authors describe the introduction of thiophosphate residues into these siRNA transcripts by incorporating thiophosphate nucleotide analogs with T7 and T3 RNA polymerase and  
10 observed that RNAs with two phosphorothioate modified bases also had substantial decreases in effectiveness as RNAi. Further, Parrish *et al.* reported that phosphorothioate modification of more than two residues greatly destabilized the RNAs *in vitro* such that interference activities could not be assayed. *Id.* at 1081. The authors also tested certain modifications at the 2'-position of the nucleotide sugar in the long siRNA transcripts and  
15 found that substituting deoxynucleotides for ribonucleotides produced a substantial decrease in interference activity, especially in the case of Uridine to Thymidine and/or Cytidine to deoxy-Cytidine substitutions. *Id.* In addition, the authors tested certain base modifications, including substituting, in sense and antisense strands of the siRNA, 4-thiouracil, 5-bromouracil, 5-iodouracil, and 3-(aminoallyl)uracil for uracil, and inosine for guanosine.  
20 Whereas 4-thiouracil and 5-bromouracil substitution appeared to be tolerated, Parrish reported that inosine produced a substantial decrease in interference activity when incorporated in either strand. Parrish also reported that incorporation of 5-iodouracil and 3-(aminoallyl)uracil in the antisense strand resulted in a substantial decrease in RNAi activity as well.

25 The use of longer dsRNA has been described. For example, Beach *et al.*, International PCT Publication No. WO 01/68836, describes specific methods for attenuating gene expression using endogenously-derived dsRNA. Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe a *Drosophila in vitro* RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications;

although Tuschl, 2001, *Chem. Biochem.*, 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due to the danger of activating interferon response. Li *et al.*, International PCT Publication No. WO 00/44914, describe the use of specific dsRNAs for attenuating the expression of certain target genes. Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646, describe certain methods for inhibiting the expression of particular genes in mammalian cells using certain dsRNA molecules. Fire *et al.*, International PCT Publication No. WO 99/32619, describe particular methods for introducing certain dsRNA molecules into cells for use in inhibiting gene expression. Plaetinck *et al.*, International PCT Publication No. WO 00/01846, describe certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific dsRNA molecules. Mello *et al.*, International PCT Publication No. WO 01/29058, describe the identification of specific genes involved in dsRNA-mediated RNAi. Deschamps Depaillette *et al.*, International PCT Publication No. WO 99/07409, describe specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Waterhouse *et al.*, International PCT Publication No. 99/53050, describe certain methods for decreasing the phenotypic expression of a nucleic acid in plant cells using certain dsRNAs. Driscoll *et al.*, International PCT Publication No. WO 01/49844, describe specific DNA constructs for use in facilitating gene silencing in targeted organisms.

Others have reported on various RNAi and gene-silencing systems. For example, Parrish *et al.*, 2000, *Molecular Cell*, 6, 1977-1087, describe specific chemically-modified siRNA constructs targeting the unc-22 gene of *C. elegans*. Grossniklaus, International PCT Publication No. WO 01/38551, describes certain methods for regulating polycomb gene expression in plants using certain dsRNAs. Churikov *et al.*, International PCT Publication No. WO 01/42443, describe certain methods for modifying genetic characteristics of an organism using certain dsRNAs. Cogoni *et al.*, International PCT Publication No. WO 01/53475, describe certain methods for isolating a *Neurospora* silencing gene and uses thereof. Reed *et al.*, International PCT Publication No. WO 01/68836, describe certain methods for gene silencing in plants. Honer *et al.*, International PCT Publication No. WO 01/70944, describe certain methods of drug screening using transgenic nematodes as Parkinson's Disease models using certain dsRNAs. Deak *et al.*, International PCT

Publication No. WO 01/72774, describe certain *Drosophila*-derived gene products that may be related to RNAi in *Drosophila*. Arndt *et al.*, International PCT Publication No. WO 01/92513 describe certain methods for mediating gene suppression by using factors that enhance RNAi. Tuschl *et al.*, International PCT Publication No. WO 02/44321, describe  
5 certain synthetic siRNA constructs. Pachuk *et al.*, International PCT Publication No. WO 00/63364, and Satishchandran *et al.*, International PCT Publication No. WO 01/04313, describe certain methods and compositions for inhibiting the function of certain polynucleotide sequences using certain dsRNAs. Echeverri *et al.*, International PCT  
10 Publication No. WO 02/38805, describe certain *C. elegans* genes identified via RNAi. Kreutzer *et al.*, International PCT Publications Nos. WO 02/055692, WO 02/055693, and EP 1144623 B1 describes certain methods for inhibiting gene expression using RNAi. Graham  
*et al.*, International PCT Publications Nos. WO 99/49029 and WO 01/70949, and AU 4037501 describe certain vector expressed siRNA molecules. Fire *et al.*, US 6,506,559, describe certain methods for inhibiting gene expression in vitro using certain long (greater  
15 than 25 nucleotide) dsRNA constructs that mediate RNAi.

Wilda *et al.*, 2002, *Oncogene*, 21, 5716, describes certain siRNA molecules targeting BCR-ABL RNA in K562 cells. BCR-ABL RNA and protein were down-regulated following siRNA treatment as shown by real-time quantitative PCR and Western blots.

## SUMMARY OF THE INVENTION

20 This invention relates to compounds, compositions, and methods useful for modulating the expression of genes, such as genes resulting from chromosomal translocation events, by RNA interference (RNAi), using short interfering nucleic acid (siNA) molecules. This invention also relates to compounds, compositions, and methods useful for modulating the  
25 expression and activity of fusion genes and transcriptional deregulation genes, or genes involved in fusion gene and transcriptional deregulation gene pathways of gene expression by RNA interference (RNAi) using small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules. In particular, the instant

invention features small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules and methods used to modulate the expression of BCR-ABL and/or ERG genes. A siNA of the invention can be unmodified or chemically-  
5 modified. A siNA of the instant invention can be chemically synthesized, expressed from a vector or enzymatically synthesized. The instant invention also features various chemically-modified synthetic short interfering nucleic acid (siNA) molecules capable of modulating fusion gene (e.g., BCR-ABL, ERG) expression or activity in cells by RNA interference (RNAi). The use of chemically-modified siNA improves various properties of native siNA  
10 molecules through increased resistance to nuclease degradation *in vivo* and/or through improved cellular uptake. Further, contrary to earlier published studies, siNA having multiple chemical modifications retains its RNAi activity. The siNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, diagnostic, target validation, genomic discovery, genetic engineering, and pharmacogenomic applications.

15 In one embodiment, the invention features one or more siNA molecules and methods that independently or in combination modulate the expression of gene(s) encoding proteins associated with chromosomal translocation events, such as BCR-ABL, TEL-AML1, EWS-FLI1, TLS-FUS, PAX3-FKHR, EWS-ERG, FUS/ERG, TLS/ERG and AML1-ETO fusion proteins. Specifically, the present invention features siNA molecules that modulate the  
20 expression of chromosomal translocation genes, for example the BCR-ABL, TEL-AML1, EWS-FLI1, TLS-FUS, PAX3-FKHR, EWS-ERG, FUS/ERG, TLS/ERG and AML1-ETO genes encoding sequences comprising those sequences referred to by GenBank Accession Nos. shown in Table I. The description below of the various aspects and embodiments of the invention is provided with reference to exemplary BCR-ABL gene. However, the  
25 various aspects and embodiments are also directed to other chromosomal translocation genes, such as TEL-AML1, EWS-FLI1, TLS-FUS, PAX3-FKHR, EWS-ERG, FUS/ERG, TLS/ERG and AML1-ETO and any other fusion gene or transcriptional deregulation genes. The various aspects and embodiments are also directed to other genes that are involved in the progression, development, or maintenance of leukemias and lymphomas. Those additional  
30 genes can be analyzed for target sites using the methods described for BCR-ABL and ERG

herein. Thus, the inhibition and the effects of such inhibition of the other genes can be performed as described herein.

In one embodiment, the invention features a siNA molecule that down-regulates expression of a BCR-ABL gene, for example, wherein the BCR-ABL gene comprises BCR-  
5 ABL encoding sequence.

In one embodiment, the invention features a siNA molecule that down-regulates expression of an ERG gene, for example, wherein the ERG gene comprises ERG encoding sequence.

In one embodiment, the invention features a siNA molecule having RNAi activity  
10 against BCR-ABL and/or ERG RNA, wherein the siNA molecule comprises a sequence complementary to any RNA having BCR-ABL and/or ERG or other BCR-ABL and/or ERG encoding sequence, such as those sequences having GenBank Accession Nos. shown in Table I. Chemical modifications as shown in Tables III and IV or otherwise described herein can be applied to any siNA construct of the invention.

15 In one embodiment, the invention features a siNA molecule having RNAi activity against polynucleotides encoding BCR-ABL and/or ERG, wherein the siNA molecule comprises a sequence complementary to any polynucleotide having BCR-ABL and/or ERG encoding sequence, such as those sequences having BCR-ABL and/or ERG GenBank Accession Nos. shown in Table I. Chemical modifications as shown in Tables III and IV  
20 or otherwise described herein can be applied to any siNA construct of the invention.

In another embodiment, the invention features a siNA molecule having RNAi activity against a BCR-ABL and/or ERG gene, wherein the siNA molecule comprises nucleotide sequence complementary to nucleotide sequence of a BCR-ABL and/or ERG gene, such as those BCR-ABL and/or ERG sequences having GenBank Accession Nos. shown in Table I.  
25 In another embodiment, a siNA molecule of the invention includes nucleotide sequence that can interact with nucleotide sequence of a BCR-ABL and/or ERG gene and thereby mediate silencing of BCR-ABL and/or ERG gene expression, for example, wherein the siNA mediates regulation of BCR-ABL and/or ERG gene expression by cellular processes that

modulate the chromatin structure of the BCR-ABL and/or ERG gene and prevent transcription of the BCR-ABL and/or ERG gene.

5 In another embodiment, the invention features a siNA molecule comprising nucleotide sequence, for example, nucleotide sequence in the antisense region of the siNA molecule that is complementary to a nucleotide sequence or portion of sequence of a BCR-ABL and/or ERG gene. In another embodiment, the invention features a siNA molecule comprising a region, for example, the antisense region of the siNA construct, complementary to a sequence or portion of sequence comprising a BCR-ABL and/or ERG gene sequence.

10 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BCR-ABL and/or ERG gene, wherein the siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is about 21 nucleotides long.

In one embodiment, a siNA molecule of the invention comprises no ribonucleotides. In another embodiment, a siNA molecule of the invention comprises ribonucleotides.

15 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the BCR-ABL and/or ERG gene, and wherein the second strand of the double-stranded siNA molecule  
20 comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the BCR-ABL and/or ERG gene.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BCR-ABL and/or ERG gene, wherein each strand of the siNA molecule comprises about 19 to about 23 nucleotides, and  
25 wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BCR-ABL and/or ERG gene, wherein the siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the BCR-ABL and/or ERG gene, and wherein the siNA further comprises a sense region, wherein the sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the BCR-ABL and/or ERG gene.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BCR-ABL and/or ERG gene, wherein the antisense region and the sense region each comprise about 19 to about 23 nucleotides, and wherein the antisense region comprises at least about 19 nucleotides that are complementary to nucleotides of the sense region.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BCR-ABL and/or ERG gene, wherein the siNA molecule comprises a sense region and an antisense region and wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of RNA encoded by the BCR-ABL and/or ERG gene and the sense region comprises a nucleotide sequence that is complementary to the antisense region.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BCR-ABL and/or ERG gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. The sense region can be connected to the antisense region via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BCR-ABL and/or ERG gene, wherein the siNA molecule comprises a sense region and an antisense region and wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide

sequence or a portion thereof of RNA encoded by the BCR-ABL and/or ERG gene and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides, 2'-deoxy purine nucleotides, or 2'-deoxy-2'-fluoro pyrimidine nucleotides.

5 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BCR-ABL and/or ERG gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule, and wherein the fragment comprising the sense region  
10 includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment comprising the sense region. In another embodiment, the terminal cap moiety is an inverted deoxy abasic moiety or glyceryl moiety. In another embodiment, each of the two fragments of the siNA molecule comprise about 21 nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic  
15 acid (siNA) molecule that down-regulates expression of a BCR-ABL and/or ERG gene, wherein the siNA molecule comprises a sense region and an antisense region and wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of RNA encoded by the BCR-ABL and/or ERG gene and the sense region comprises a nucleotide sequence that is complementary to the antisense region,  
20 and wherein the purine nucleotides present in the antisense region comprise 2'-deoxy- purine nucleotides. In another embodiment, the antisense region comprises a phosphorothioate internucleotide linkage at the 3' end of the antisense region. In another embodiment, the antisense region comprises a glyceryl modification at the 3' end of the antisense region.

In one embodiment, the invention features a double-stranded short interfering nucleic  
25 acid (siNA) molecule that down-regulates expression of a BCR-ABL and/or ERG gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule, and wherein about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of



the siNA molecule and wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In another embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule are 2'-deoxy-pyrimidines, such as 2'-deoxy-thymidine. In another  
5 embodiment, all 21 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the BCR-ABL and/or ERG gene. In another embodiment, 21 nucleotides of the antisense region are base-paired to the nucleotide  
10 sequence or a portion thereof of the RNA encoded by the BCR-ABL and/or ERG gene. In another embodiment, the 5'-end of the fragment comprising said antisense region optionally includes a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a BCR-ABL and/or ERG RNA sequence  
15 (e.g., wherein said target RNA sequence is encoded by a BCR-ABL and/or ERG gene), wherein the siNA molecule comprises no ribonucleotides and wherein each strand of the double-stranded siNA molecule is about 21 nucleotides long.

In one embodiment, the invention features a medicament comprising a siNA molecule of the invention.

20 In one embodiment, the invention features an active ingredient comprising a siNA molecule of the invention.

In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule to down-regulate expression of a BCR-ABL and/or ERG gene, wherein the siNA molecule comprises one or more chemical modifications and  
25 each strand of the double-stranded siNA is about 21 nucleotides long.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises

nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule  
5 comprises a sugar modification.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG  
10 RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence of the antisense strand of the double-stranded siNA molecule is complementary to the nucleotide sequence of the  
15 BCR-ABL and/or ERG RNA or a portion thereof which encodes an protein or a portion thereof.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises  
20 nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein each strand of the siNA molecule comprises  
25 about 19 to about 29 nucleotides, and wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises

nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the siNA molecule is assembled from two oligonucleotide fragments wherein one fragment comprises the nucleotide sequence of the antisense strand of the siNA molecule and a second fragment comprises nucleotide sequence of the sense region of the siNA molecule.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the sense strand is connected to the antisense strand via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein purine nucleotides present in the sense region are 2'-deoxy purine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one

of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein  
5 a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the sense strand comprises a 3'-end and a 5'-end, and wherein a terminal cap moiety (e.g., an inverted deoxy abasic moiety) is present at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand.

In one embodiment, the invention features a double-stranded short interfering nucleic  
10 acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein  
15 a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the antisense strand comprises one or more 2'-deoxy-2'-fluoro pyrimidine nucleotides and one or more 2'-O-methyl purine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic  
20 acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein  
25 a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein any purine nucleotides present in the antisense strand are 2'-O-methyl purine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one

of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein  
5 a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the antisense strand comprises a phosphorothioate internucleotide linkage at the 3' end of the antisense strand.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one  
10 of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein  
15 a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the antisense strand comprises a glyceryl modification at the 3' end.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one  
20 of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein  
25 a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein each of the two strands of the siNA molecule comprises 21 nucleotides. In another embodiment, about 19 nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule and wherein at least two 3' terminal nucleotides of each strand of the siNA molecule are not base-paired to the nucleotides of the other strand of the siNA molecule. In another embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA

molecule are 2'-deoxy-pyrimidines, such as 2'-deoxy-thymidine. In another embodiment, each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule. In another embodiment, about 19 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the BCR-ABL and/or ERG  
5 RNA or a portion thereof. In another embodiment, 21 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the BCR-ABL and/or ERG RNA or a portion thereof.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one  
10 of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule  
15 comprises a sugar modification, and wherein the 5'-end of the antisense strand optionally includes a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises  
20 nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence or a portion thereof of  
25 the antisense strand is complementary to a nucleotide sequence of the 5'-untranslated region or a portion thereof of the BCR-ABL and/or ERG RNA.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises

nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule  
5 comprises a sugar modification, and wherein the nucleotide sequence or a portion thereof of the antisense strand is complementary to a nucleotide sequence of the BCR-ABL and/or ERG RNA or a portion thereof that is present in the BCR-ABL and/or ERG RNA.

In one embodiment, the invention features a pharmaceutical composition comprising a siNA molecule of the invention in an acceptable carrier or diluent.

10 In one embodiment, the invention features a medicament comprising an siNA molecule of the invention.

In one embodiment, the invention features an active ingredient comprising an siNA molecule of the invention.

In one embodiment, the invention features the use of a double-stranded short  
15 interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the  
20 antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

In one embodiment, the antisense region of BCR-ABL siRNA constructs can comprise a sequence complementary to sequence having any of SEQ ID NOs. 1-263, 527-845, 1165-1182, 1201-1218 or 1589-1596. In another embodiment, the antisense region can also  
25 comprise sequence having any of SEQ ID NOs. 264-526, 846-1164, 1183-1200, 1219-1236, 1605-1608, 1613-1616, 1621-1624, 1629-1631, 1637-1640, 1645-1648, 1685, 1687, 1689, 1691, 1693, or 1694. In another embodiment, the sense region of BCR-ABL siRNA constructs can comprise sequence having any of SEQ ID NOs. 1-263, 527-845, 1165-1182,

1201-1218, 1589-1596, 1601-1604, 1609-1612, 1617-1620, 1625-1628, 1632-1636, 1641-1644, 1684, 1686, 1688, 1690, or 1692. The sense region can comprise a sequence of SEQ ID NO. 1673 and the antisense region can comprise a sequence of SEQ ID NO. 1674. The sense region can comprise a sequence of SEQ ID NO. 1675 and the antisense region can  
5 comprise a sequence of SEQ ID NO. 1676. The sense region can comprise a sequence of SEQ ID NO. 1677 and the antisense region can comprise a sequence of SEQ ID NO. 1678. The sense region can comprise a sequence of SEQ ID NO. 1679 and the antisense region can comprise a sequence of SEQ ID NO. 1680. The sense region can comprise a sequence of SEQ ID NO. 1681 and the antisense region can comprise a sequence of SEQ ID NO. 1682.  
10 The sense region can comprise a sequence of SEQ ID NO. 1679 and the antisense region can comprise a sequence of SEQ ID NO. 1683.

In one embodiment, the antisense region of ERG siRNA constructs can comprise a sequence complementary to sequence having any of SEQ ID NOs. 1237-1412 or 1597-1600. In another embodiment, the antisense region can also comprise sequence having any of SEQ  
15 ID NOs. 1413-1588, 1653-1656, 1661-1664, 1669-1672, 1696, 1698, 1700, 1702, 1704, or 1705. In another embodiment, the sense region of ERG siRNA constructs can comprise sequence having any of SEQ ID NOs. 1237-1412, 1597-1600, 1649-1652, 1657-1660, 1665-1668, 1695, 1697, 1699, 1701, or 1703. The sense region can comprise a sequence of SEQ ID NO. 1673 and the antisense region can comprise a sequence of SEQ ID NO. 1674. The  
20 sense region can comprise a sequence of SEQ ID NO. 1675 and the antisense region can comprise a sequence of SEQ ID NO. 1676. The sense region can comprise a sequence of SEQ ID NO. 1677 and the antisense region can comprise a sequence of SEQ ID NO. 1678. The sense region can comprise a sequence of SEQ ID NO. 1679 and the antisense region can comprise a sequence of SEQ ID NO. 1680. The sense region can comprise a sequence of  
25 SEQ ID NO. 1681 and the antisense region can comprise a sequence of SEQ ID NO. 1682. The sense region can comprise a sequence of SEQ ID NO. 1679 and the antisense region can comprise a sequence of SEQ ID NO. 1683.

In one embodiment, a siNA molecule of the invention comprises any of SEQ ID NOs. 1-1705. The sequences shown in SEQ ID NOs: 1-1705 are not limiting. A siNA molecule of



the invention can comprise any contiguous BCR-ABL and/or ERG sequence (e.g., about 19 to about 25, or about 19, 20, 21, 22, 23, 24 or 25 contiguous BCR-ABL and/or ERG nucleotides).

5 In yet another embodiment, the invention features a siNA molecule comprising a sequence, for example, the antisense sequence of the siNA construct, complementary to a sequence or portion of sequence comprising sequence represented by GenBank Accession Nos. shown in **Table I**. Chemical modifications in **Tables III and IV** and described herein can be applied to any siNA construct of the invention.

10 In one embodiment of the invention a siNA molecule comprises an antisense strand having about 19 to about 29 nucleotides, wherein the antisense strand is complementary to a RNA sequence encoding a BCR-ABL and/or ERG protein, and wherein said siNA further comprises a sense strand having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29) nucleotides, and wherein said sense strand and said antisense strand are distinct nucleotide sequences with at least about 19 complementary nucleotides.

15 In another embodiment of the invention a siNA molecule of the invention comprises an antisense region having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29) nucleotides, wherein the antisense region is complementary to a RNA sequence encoding a BCR-ABL and/or ERG protein, and wherein said siNA further comprises a sense region having about 19 to about 29 nucleotides, wherein said sense region and said antisense  
20 region comprise a linear molecule with at least about 19 complementary nucleotides.

In one embodiment of the invention a siNA molecule comprises an antisense strand comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a BCR-ABL and/or ERG protein. The siNA further comprises a sense strand, wherein said sense strand comprises a nucleotide sequence of a BCR-ABL  
25 and/or ERG gene or a portion thereof.

In another embodiment, a siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a BCR-ABL and/or ERG protein. The siNA molecule further comprises a sense

region, wherein said sense region comprises a nucleotide sequence of a BCR-ABL and/or ERG gene or a portion thereof.

In one embodiment, a siNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a BCR-ABL and/or ERG gene. Because BCR-ABL and/or ERG genes can share some degree of sequence homology with each other, siNA molecules can be designed to target a class of BCR-ABL and/or ERG genes (and associated receptor or ligand genes) or alternately specific BCR-ABL and/or ERG genes by selecting sequences that are either shared amongst different BCR-ABL and/or ERG targets or alternatively that are unique for a specific BCR-ABL and/or ERG target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved regions of BCR-ABL and/or ERG RNA sequence having homology between several BCR-ABL and/or ERG receptor genes so as to target several BCR-ABL and/or ERG genes (e.g., different BCR-ABL and/or ERG subunits, isoforms, splice variants, mutant genes etc.) with one siNA molecule. In another embodiment, the siNA molecule can be designed to target a sequence that is unique to a specific BCR-ABL and/or ERG RNA sequence due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

In one embodiment, nucleic acid molecules of the invention that act as mediators of the RNA interference gene silencing response are double-stranded nucleic acid molecules. In another embodiment, the siNA molecules of the invention consist of duplexes containing about 19 base pairs between oligonucleotides comprising about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24 or 25) nucleotides. In yet another embodiment, siNA molecules of the invention comprise duplexes with overhanging ends of about about 1 to about 3 (e.g., about 1, 2, or 3) nucleotides, for example, about 21-nucleotide duplexes with about 19 base pairs and 3'-terminal mononucleotide, dinucleotide, or trinucleotide overhangs.

In one embodiment, the invention features one or more chemically-modified siNA constructs having specificity for BCR-ABL and/or ERG expressing nucleic acid molecules, such as RNA encoding a BCR-ABL and/or ERG protein. Non-limiting examples of such chemical modifications include without limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides,

"universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, and terminal glyceryl and/or inverted deoxy abasic residue incorporation. These chemical modifications, when used in various siNA constructs, are shown to preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds. Furthermore, 5 contrary to the data published by Parrish *et al.*, *supra*, applicant demonstrates that multiple (greater than one) phosphorothioate substitutions are well-tolerated and confer substantial increases in serum stability for modified siNA constructs.

In one embodiment, a siNA molecule of the invention comprises modified nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides can be used to 10 improve *in vitro* or *in vivo* characteristics such as stability, activity, and/or bioavailability. For example, a siNA molecule of the invention can comprise modified nucleotides as a percentage of the total number of nucleotides present in the siNA molecule. As such, a siNA molecule of the invention can generally comprise about 5% to 100% modified nucleotides (e.g., 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 15 80%, 85%, 90%, 95% or 100% modified nucleotides). The actual percentage of modified nucleotides present in a given siNA molecule will depend on the total number of nucleotides present in the siNA. If the siNA molecule is single stranded, the percent modification can be based upon the total number of nucleotides present in the single stranded siNA molecules. Likewise, if the siNA molecule is double stranded, the percent modification can be based 20 upon the total number of nucleotides present in the sense strand, antisense strand, or both the sense and antisense strands.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises 25 nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, the BCR-ABL and/or ERG gene

encodes sequence comprising Genbank Accession number NM\_004327 (BCR). In one embodiment, the BCR-ABL and/or ERG gene encodes sequence comprising Genbank Accession number NM\_005157 (ABL).

5 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein  
10 a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence of the antisense strand of the double-stranded siNA molecule is complementary to the nucleotide sequence of the BCR-ABL and/or ERG RNA or a portion thereof which encodes an protein or a portion thereof.

15 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein  
20 a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein each strand of the siNA molecule comprises about 19 to about 29 nucleotides, and wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.

25 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide

sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the siNA molecule is assembled from two oligonucleotide fragments wherein one fragment comprises the nucleotide sequence of the antisense strand of the siNA molecule and a second fragment comprises nucleotide sequence of the sense region of the siNA molecule.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the sense strand is connected to the antisense strand via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein purine nucleotides present in the sense region are 2'-deoxy purine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG

RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the sense strand comprises a 3'-end and a 5'-end, and wherein a terminal cap moiety (e.g., an inverted deoxy abasic moiety) is present at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the antisense strand comprises one or more 2'-deoxy-2'-fluoro pyrimidine nucleotides and one or more 2'-O-methyl purine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein any purine nucleotides present in the antisense strand are 2'-O-methyl purine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG

RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the antisense strand comprises a  
5 phosphorothioate internucleotide linkage at the 3' end of the antisense strand.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG  
10 RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the antisense strand comprises a glyceryl modification at the 3' end.

15 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG  
20 RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein each of the two strands of the siNA molecule comprises 21 nucleotides. In another embodiment, about 19 nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the  
25 siNA molecule and wherein at least two 3' terminal nucleotides of each strand of the siNA molecule are not base-paired to the nucleotides of the other strand of the siNA molecule. In another embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule are 2'-deoxy-pyrimidines, such as 2'-deoxy-thymidine. In another embodiment, each strand of the siNA molecule are base-paired to the complementary nucleotides of the

other strand of the siNA molecule. In another embodiment, about 19 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the RNA or a portion thereof of BCR-ABL and/or ERG RNA. In another embodiment, 21 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the BCR-ABL and/or ERG RNA or a  
5 portion thereof.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG  
10 RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the 5'-end of the antisense strand optionally includes a phosphate group.

15 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide  
20 sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence or a portion thereof of the antisense strand is complementary to a nucleotide sequence of the 5'-untranslated region or a portion thereof of the BCR-ABL and/or ERG RNA.

25 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide



sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence or a portion thereof of the antisense strand is complementary to a nucleotide sequence of the BCR-ABL and/or ERG  
5 RNA or a portion thereof that is present in the BCR-ABL and/or ERG RNA.

In one embodiment, the invention features a pharmaceutical composition comprising a siNA molecule of the invention in an acceptable carrier or diluent.

In one embodiment, the invention features a medicament comprising an siNA molecule of the invention.

10 In one embodiment, the invention features an active ingredient comprising an siNA molecule of the invention.

In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a BCR-ABL and/or ERG gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand  
15 which comprises nucleotide sequence that is complementary to nucleotide sequence of BCR-ABL and/or ERG RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

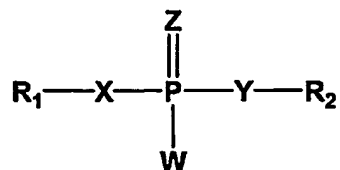
20 In a non-limiting example, the introduction of chemically-modified nucleotides into nucleic acid molecules provides a powerful tool in overcoming potential limitations of *in vivo* stability and bioavailability inherent to native RNA molecules that are delivered exogenously. For example, the use of chemically-modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since  
25 chemically-modified nucleic acid molecules tend to have a longer half-life in serum. Furthermore, certain chemical modifications can improve the bioavailability of nucleic acid molecules by targeting particular cells or tissues and/or improving cellular uptake of the nucleic acid molecule. Therefore, even if the activity of a chemically-modified nucleic acid

molecule is reduced as compared to a native nucleic acid molecule, for example, when compared to an all-RNA nucleic acid molecule, the overall activity of the modified nucleic acid molecule can be greater than that of the native molecule due to improved stability and/or delivery of the molecule. Unlike native unmodified siNA, chemically-modified siNA can  
5 also minimize the possibility of activating interferon activity in humans.

The antisense region of a siNA molecule of the invention can comprise a phosphorothioate internucleotide linkage at the 3'-end of the antisense region. The antisense region can comprise about one to about five phosphorothioate internucleotide linkages at the 5'-end of the antisense region. The 3'-terminal nucleotide overhangs of a siNA molecule of  
10 the invention can comprise ribonucleotides or deoxyribonucleotides that are chemically-modified at a nucleic acid sugar, base, or backbone. The 3'-terminal nucleotide overhangs can comprise one or more universal base ribonucleotides. The 3'-terminal nucleotide overhangs can comprise one or more acyclic nucleotides.

One embodiment of the invention provides an expression vector comprising a nucleic  
15 acid sequence encoding at least one siNA molecule of the invention in a manner that allows expression of the nucleic acid molecule. Another embodiment of the invention provides a mammalian cell comprising such an expression vector. The mammalian cell can be a human cell. The siNA molecule of the expression vector can comprise a sense region and an antisense region. The antisense region can comprise sequence complementary to a RNA or  
20 DNA sequence encoding BCR-ABL and/or ERG and the sense region can comprise sequence complementary to the antisense region. The siNA molecule can comprise two distinct strands having complementary sense and antisense regions. The siNA molecule can comprise a single strand having complementary sense and antisense regions.

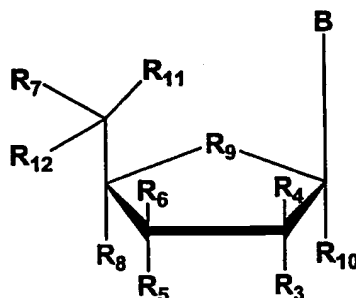
In one embodiment, the invention features a chemically-modified short interfering  
25 nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a BCR-ABL and/or ERG inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides comprising a backbone modified internucleotide linkage having Formula I:



wherein each R1 and R2 is independently any nucleotide, non-nucleotide, or polynucleotide which can be naturally-occurring or chemically-modified, each X and Y is independently O, S, N, alkyl, or substituted alkyl, each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl, and wherein W, X, Y, and Z are optionally not all O.

The chemically-modified internucleotide linkages having Formula I, for example, wherein any Z, W, X, and/or Y independently comprises a sulphur atom, can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) chemically-modified internucleotide linkages having Formula I at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified internucleotide linkages having Formula I at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In another embodiment, a siNA molecule of the invention having internucleotide linkage(s) of Formula I also comprises a chemically-modified nucleotide or non-nucleotide having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a BCR-ABL and/or ERG inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula II:

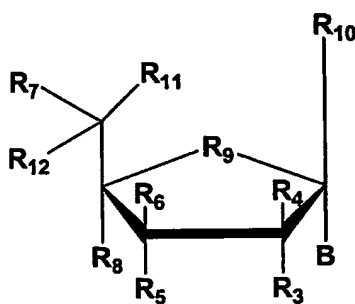


wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula II can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula II at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5

or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end of the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a BCR-ABL and/or ERG inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula III:



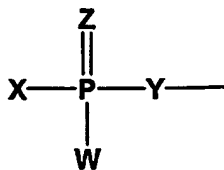
wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be employed to be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other

non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula III can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide(s) or non-nucleotide(s) of Formula III at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end of the sense strand, the antisense strand, or both strands.

In another embodiment, a siNA molecule of the invention comprises a nucleotide having Formula II or III, wherein the nucleotide having Formula II or III is in an inverted configuration. For example, the nucleotide having Formula II or III is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a BCR-ABL and/or ERG inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a 5'-terminal phosphate group having Formula IV:



wherein each X and Y is independently O, S, N, alkyl, substituted alkyl, or alkylhalo; wherein each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, or alkylhalo; and wherein W, X, Y and Z are not all O.

In one embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand, for example, a strand complementary to a target RNA, wherein the siNA molecule comprises an all RNA siNA molecule. In another embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand wherein the siNA molecule also comprises about 1 to about 3 (e.g., about 1, 2, or 3) nucleotide 3'-terminal nucleotide overhangs having about 1 to about 4 (e.g., about 1, 2, 3, or 4) deoxyribonucleotides on the 3'-end of one or both strands. In another embodiment, a 5'-terminal phosphate group having Formula IV is present on the target-complementary strand of a siNA molecule of the invention, for example a siNA molecule having chemical modifications having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against a BCR-ABL and/or ERG inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more phosphorothioate internucleotide linkages. For example, in a non-limiting example, the invention features a chemically-modified short interfering nucleic acid (siNA) having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in one siNA strand. In yet another embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) individually having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in both siNA strands. The phosphorothioate internucleotide linkages can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3,

4, 5, or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a siNA molecule, wherein the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the sense strand comprises about 1 to about 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) universal



base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5 or more, for example about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a siNA molecule, wherein the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3' and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule having about 1 to about 5, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages in each strand of the siNA molecule.

In another embodiment, the invention features a siNA molecule comprising 2'-5' internucleotide linkages. The 2'-5' internucleotide linkage(s) can be at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of one or both siNA sequence strands. In addition, the 2'-5' internucleotide linkage(s) can be present at various other positions within one or both siNA sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a purine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage.

In another embodiment, a chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified, wherein each strand is about 18 to about 27 (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27) nucleotides in length, wherein the duplex has about 18 to about 23 (e.g., about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the chemical modification comprises a structure having any of Formulae I-VII. For example, an exemplary chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein each strand consists of about 21 nucleotides, each having a 2-nucleotide 3'-terminal nucleotide overhang, and wherein the duplex has about 19 base pairs.

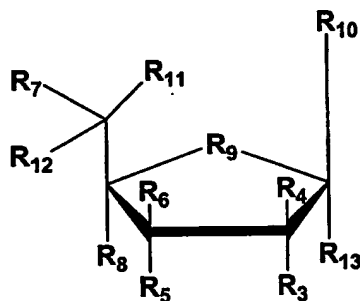
In another embodiment, a siNA molecule of the invention comprises a single stranded hairpin structure, wherein the siNA is about 36 to about 70 (e.g., about 36, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 18 to about 23 (e.g., about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the siNA can include a chemical modification comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 42 to about 50 (e.g., about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 19 base pairs and a 2-nucleotide 3'-terminal nucleotide overhang. In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. For example, a linear hairpin siNA molecule of the invention is designed such that degradation of the loop portion of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In another embodiment, a siNA molecule of the invention comprises a circular nucleic acid molecule, wherein the siNA is about 38 to about 70 (e.g., about 38, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 18 to about 23 (e.g., about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the siNA can include a chemical modification, which comprises a structure having any of Formulae I-VII or any combination thereof. For example, an

exemplary chemically-modified siNA molecule of the invention comprises a circular oligonucleotide having about 42 to about 50 (*e.g.*, about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the circular oligonucleotide forms a dumbbell shaped structure having about 19 base pairs and 2 loops.

In another embodiment, a circular siNA molecule of the invention contains two loop motifs, wherein one or both loop portions of the siNA molecule is biodegradable. For example, a circular siNA molecule of the invention is designed such that degradation of the loop portions of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In one embodiment, a siNA molecule of the invention comprises at least one (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) abasic moiety, for example a compound having Formula V:

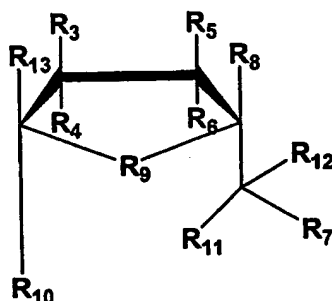


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wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2.

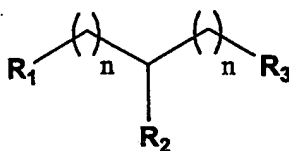
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In one embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) inverted abasic moiety, for example a compound having Formula VI:



- 5 wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2, and either R2, R3, R8 or R13  
10 serve as points of attachment to the siNA molecule of the invention.

- In another embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) substituted polyalkyl moieties, for example a  
15 compound having Formula VII:



- wherein each n is independently an integer from 1 to 12, each R1, R2 and R3 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl,  
20

ONO<sub>2</sub>, NO<sub>2</sub>, N<sub>3</sub>, NH<sub>2</sub>, aminoalkyl, aminoacid, aminoacyl, ONH<sub>2</sub>, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkdylamino, substituted silyl, or a group having Formula I, and R<sub>1</sub>, R<sub>2</sub> or R<sub>3</sub> serves as points of attachment to the siNA molecule of the invention.

5        In another embodiment, the invention features a compound having Formula VII, wherein R<sub>1</sub> and R<sub>2</sub> are hydroxyl (OH) groups, n = 1, and R<sub>3</sub> comprises O and is the point of attachment to the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both strands of a double-stranded siNA molecule of the invention or to a single-stranded siNA molecule of the invention. This modification is referred to herein as "glyceryl" (for example modification 6  
10 in Figure 10).

      In another embodiment, a moiety having any of Formula V, VI or VII of the invention is at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of a siNA molecule of the invention. For example, a moiety having Formula V, VI or VII can be present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense strand, the sense strand, or both antisense and  
15 sense strands of the siNA molecule. In addition, a moiety having Formula VII can be present at the 3'-end or the 5'-end of a hairpin siNA molecule as described herein.

      In another embodiment, a siNA molecule of the invention comprises an abasic residue having Formula V or VI, wherein the abasic residue having Formula VI or VI is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-  
20 end, or both of the 3' and 5'-ends of one or both siNA strands.

      In one embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) locked nucleic acid (LNA) nucleotides, for example at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

25        In another embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) acyclic nucleotides, for example at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises a sense region, where any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all  
5 pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

10 In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises a sense region, where any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality  
15 of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-  
20 deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises an antisense region, where any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein  
25 all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine

nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises an antisense region, where any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said antisense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention, wherein the chemically-modified siNA comprises an antisense region, where any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against a BCR-ABL and/or ERG inside a cell or reconstituted *in vitro* system, wherein the chemically-modified siNA comprises a sense region, where one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro



pyrimidine nucleotides), and where one or more purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and inverted deoxy abasic modifications that are optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense region, the sense region optionally further comprising a 3'-terminal overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxyribonucleotides; and wherein the chemically-modified short interfering nucleic acid molecule comprises an antisense region, where one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein one or more purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the antisense region optionally further comprising a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides, wherein the overhang nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4 ) phosphorothioate internucleotide linkages. Non-limiting examples of these chemically-modified siNAs are shown in Figures 4 and 5 and Tables III and IV herein.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against a BCR-ABL and/or ERG inside a cell or reconstituted *in vitro* system, wherein the siNA comprises a sense region, where one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and where one or more purine nucleotides present in the sense region are purine ribonucleotides (e.g., wherein all purine nucleotides are purine ribonucleotides or alternately a plurality of purine

nucleotides are purine ribonucleotides), and inverted deoxy abasic modifications that are optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense region, the sense region optionally further comprising a 3'-terminal overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxyribonucleotides; and wherein the siNA comprises an  
5 antisense region, where one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides  
10 are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the antisense region optionally further comprising a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or  
15 4) 2'-deoxynucleotides, wherein the overhang nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4 ) phosphorothioate internucleotide linkages. Non-limiting examples of these chemically-modified siNAs are shown in Figures 4 and 5 and Tables III and IV herein.

In one embodiment, the invention features a chemically-modified short interfering  
20 nucleic acid (siNA) molecule of the invention capable of mediating RNA interference (RNAi) against a BCR-ABL and/or ERG inside a cell or reconstituted *in vitro* system, wherein the chemically-modified siNA comprises a sense region, where one or more pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine  
25 nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and for example where one or more purine nucleotides present in the sense region are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides (e.g., wherein all purine nucleotides are selected from the group  
30 consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl

nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides or alternately a plurality of purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides), and wherein inverted deoxy abasic modifications are optionally present

5 at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense region, the sense region optionally further comprising a 3'-terminal overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxyribonucleotides; and wherein the chemically-modified short interfering nucleic acid molecule comprises an antisense region, where one or more pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides

10 (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein one or more purine nucleotides present in the antisense region are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides

15 (e.g., wherein all purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides or alternately a plurality of purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides),

20 and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the antisense region optionally further comprising a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides, wherein the overhang nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4)

25 phosphorothioate internucleotide linkages.

In another embodiment, any modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides.

30 For example, the invention features siNA molecules including modified nucleotides having a

Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, are resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi. Non-limiting examples of nucleotides having a northern configuration include locked nucleic acid (LNA) nucleotides (e.g., 2'-O,4'-C-methylene-(D-ribofuranosyl) nucleotides); 2'-methoxyethoxy (MOE) nucleotides; 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy-2'-chloro nucleotides, 2'-azido nucleotides, and 2'-O-methyl nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid molecule (siNA) capable of mediating RNA interference (RNAi) against a BCR-ABL and/or ERG inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a conjugate covalently attached to the chemically-modified siNA molecule. In another embodiment, the conjugate is covalently attached to the chemically-modified siNA molecule via a biodegradable linker. In one embodiment, the conjugate molecule is attached at the 3'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In another embodiment, the conjugate molecule is attached at the 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In yet another embodiment, the conjugate molecule is attached both the 3'-end and 5'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule, or any combination thereof. In one embodiment, a conjugate molecule of the invention comprises a molecule that facilitates delivery of a chemically-modified siNA molecule into a biological system, such as a cell. In another embodiment, the conjugate molecule attached to the chemically-modified siNA molecule is a poly ethylene glycol, human serum albumin, or a ligand for a cellular receptor that can mediate cellular uptake. Examples of specific conjugate molecules contemplated by the instant invention that can be attached to chemically-modified siNA molecules are described in Vargeese *et al.*, U.S. Serial No. 10/201,394, incorporated by reference herein. The type of conjugates used and the extent of

conjugation of siNA molecules of the invention can be evaluated for improved pharmacokinetic profiles, bioavailability, and/or stability of siNA constructs while at the same time maintaining the ability of the siNA to mediate RNAi activity. As such, one skilled in the art can screen siNA constructs that are modified with various conjugates to determine  
5 whether the siNA conjugate complex possesses improved properties while maintaining the ability to mediate RNAi, for example in animal models as are generally known in the art.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule of the invention, wherein the siNA further comprises a nucleotide, non-nucleotide, or mixed nucleotide/non-nucleotide linker that joins the sense region of the siNA to the  
10 antisense region of the siNA. In one embodiment, a nucleotide linker of the invention can be a linker of  $\geq 2$  nucleotides in length, for example 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length. In another embodiment, the nucleotide linker can be a nucleic acid aptamer. By "aptamer" or "nucleic acid aptamer" as used herein is meant a nucleic acid molecule that binds specifically to a target molecule wherein the nucleic acid molecule has sequence that  
15 comprises a sequence recognized by the target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to a target molecule where the target molecule does not naturally bind to a nucleic acid. The target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand-binding domain of a protein, thereby preventing interaction of the naturally occurring ligand with the protein.  
20 This is a non-limiting example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. (See, for example, Gold *et al.*, 1995, *Annu. Rev. Biochem.*, 64, 763; Brody and Gold, 2000, *J. Biotechnol.*, 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.*, 2, 100; Kusser, 2000, *J. Biotechnol.*, 74, 27; Hermann and Patel, 2000, *Science*, 287, 820; and Jayasena, 1999, *Clinical Chemistry*, 45, 1628.)

25 In yet another embodiment, a non-nucleotide linker of the invention comprises abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, polyhydrocarbon, or other polymeric compounds (e.g. polyethylene glycols such as those having between 2 and 100 ethylene glycol units). Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, 18:6353 and *Nucleic Acids Res.* 1987, 15:3113; Cload and

Schepartz, *J. Am. Chem. Soc.* 1991, 113:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, 113:5109; Ma *et al.*, *Nucleic Acids Res.* 1993, 21:2585 and *Biochemistry* 1993, 32:1751; Durand *et al.*, *Nucleic Acids Res.* 1990, 18:6353; McCurdy *et al.*, *Nucleosides & Nucleotides* 1991, 10:287; Jschke *et al.*, *Tetrahedron Lett.* 1993, 34:301; Ono *et al.*,  
5 *Biochemistry* 1991, 30:9914; Arnold *et al.*, International Publication No. WO 89/02439; Usman *et al.*, International Publication No. WO 95/06731; Dudycz *et al.*, International Publication No. WO 95/11910 and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, 113:4000, all hereby incorporated by reference herein. A "non-nucleotide" further means any group or compound that can be incorporated into a nucleic acid chain in the place of one or more  
10 nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine, for example at the C1 position of the sugar.

In one embodiment, the invention features a short interfering nucleic acid (siNA)  
15 molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted in vitro system, wherein one or both strands of the siNA molecule that are assembled from two separate oligonucleotides do not comprise any ribonucleotides. For example, a siNA molecule can be assembled from a single oligonucleotide where the sense and antisense regions of the siNA comprise separate oligonucleotides not having any ribonucleotides (e.g.,  
20 nucleotides having a 2'-OH group) present in the oligonucleotides. In another example, a siNA molecule can be assembled from a single oligonucleotide where the sense and antisense regions of the siNA are linked or circularized by a nucleotide or non-nucleotide linker as described herein, wherein the oligonucleotide does not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotide. Applicant has surprisingly  
25 found that the presense of ribonucleotides (e.g., nucleotides having a 2'-hydroxyl group) within the siNA molecule is not required or essential to support RNAi activity. As such, in one embodiment, all positions within the siNA can include chemically modified nucleotides and/or non-nucleotides such as nucleotides and or non-nucleotides having Formula I, II, III, IV, V, VI, or VII or any combination thereof to the extent that the ability of the siNA  
30 molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group and a 3'-terminal phosphate group (e.g., a 2', 3'-cyclic phosphate). In another embodiment, the single stranded siNA molecule of the invention comprises about 19 to about 29 nucleotides. In yet another embodiment, the single stranded siNA molecule of the invention comprises one or more chemically modified nucleotides or non-nucleotides described herein. For example, all the positions within the siNA molecule can include chemically-modified nucleotides such as nucleotides having any of Formulae I-VII, or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the siNA optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4 ) phosphorothioate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the  
5 siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-deoxy purine nucleotides (e.g., wherein all  
10 purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the siNA optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the  
15 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4 ) phosphorothioate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the  
20 siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine  
25 nucleotides present in the antisense region are locked nucleic acid (LNA) nucleotides (e.g., wherein all purine nucleotides are LNA nucleotides or alternately a plurality of purine nucleotides are LNA nucleotides), and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the siNA optionally further comprising  
30 about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the



siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4 ) phosphorothioate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted in vitro system, wherein the siNA molecule comprises a single stranded polynucleotide having complementarity to a target nucleic acid sequence, and wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine nucleotides present in the antisense region are 2'-methoxyethyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-methoxyethyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-methoxyethyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence, the siNA optionally further comprising about 1 to about 4 (e.g., about 1, 2, 3, or 4) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, or 4 ) phosphorothioate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group.

In another embodiment, any modified nucleotides present in the single stranded siNA molecules of the invention comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example. Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the single stranded siNA molecules of the invention are preferably resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi.

In one embodiment, siNA molecules of the invention are used as reagents in ex vivo gene therapy applications. For example, siNA reagents are introduced into tissue or cells that are transplanted into a subject for therapeutic effect. The cells and/or tissue can be derived from an organism or subject that later receives the explant, or can be derived from another organism or subject prior to transplantation. The siNA molecules can be used to modulate the expression of one or more genes in the cells or tissue, such that the cells or tissue obtain a desired phenotype or are able to perform a function when transplanted in vivo. In one embodiment, the invention features a method for modulating the expression of a BCR-ABL and/or ERG gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BCR-ABL and/or ERG gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the BCR-ABL and/or ERG gene in the cell.

In one embodiment, the invention features a method for modulating the expression of a BCR-ABL and/or ERG gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BCR-ABL and/or ERG gene and wherein the sense strand sequence of the siNA comprises a sequence identical to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the BCR-ABL and/or ERG gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one BCR-ABL and/or ERG gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BCR-ABL and/or ERG genes; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the BCR-ABL and/or ERG genes in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one BCR-ABL and/or ERG gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA

strands comprises a sequence complementary to RNA of the BCR-ABL and/or ERG gene and wherein the sense strand sequence of the siNA comprises a sequence identical to the sequence of the target RNA; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the BCR-ABL and/or ERG genes in the  
5 cell.

In one embodiment, the invention features a method of modulating the expression of a BCR-ABL and/or ERG gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BCR-ABL and/or ERG gene;  
10 and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the BCR-ABL  
15 and/or ERG gene in that organism.

In one embodiment, the invention features a method of modulating the expression of a BCR-ABL and/or ERG gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BCR-ABL and/or ERG gene  
20 and wherein the sense strand sequence of the siNA comprises a sequence identical to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue  
25 was derived from or into another organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG gene in that organism.

In another embodiment, the invention features a method of modulating the expression of more than one BCR-ABL and/or ERG gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein

one of the siNA strands comprises a sequence complementary to RNA of the BCR-ABL and/or ERG genes; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG genes in the tissue explant. In another embodiment, the method  
5 further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG genes in that organism.

In one embodiment, the invention features a method of modulating the expression of a BCR-ABL and/or ERG gene in an organism comprising: (a) synthesizing a siNA molecule  
10 of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BCR-ABL and/or ERG gene; and (b) introducing the siNA molecule into the organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG gene in the organism.

In another embodiment, the invention features a method of modulating the expression  
15 of more than one BCR-ABL and/or ERG gene in an organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the BCR-ABL and/or ERG genes; and (b) introducing the siNA molecules into the organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG genes in the organism.

In one embodiment, the invention features a method for modulating the expression of a BCR-ABL and/or ERG gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single  
20 stranded sequence having complementarity to RNA of the BCR-ABL and/or ERG gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the BCR-ABL and/or ERG gene in the cell.  
25

In another embodiment, the invention features a method for modulating the expression of more than one BCR-ABL and/or ERG gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA

comprises a single stranded sequence having complementarity to RNA of the BCR-ABL and/or ERG gene; and (b) contacting the siNA molecule with a cell in vitro or in vivo under conditions suitable to modulate the expression of the BCR-ABL and/or ERG genes in the cell.

5           In one embodiment, the invention features a method of modulating the expression of a BCR-ABL and/or ERG gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the BCR-ABL and/or ERG gene; and (b) contacting the siNA molecule with a cell of the tissue explant derived from a  
10 particular organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG gene in that organism.

15           In another embodiment, the invention features a method of modulating the expression of more than one BCR-ABL and/or ERG gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the BCR-ABL and/or ERG gene; and (b) introducing the siNA molecules into a cell of the tissue  
20 explant derived from a particular organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG genes in that organism.

25           In one embodiment, the invention features a method of modulating the expression of a BCR-ABL and/or ERG gene in an organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the BCR-ABL and/or ERG gene; and

(b) introducing the siNA molecule into the organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG gene in the organism.

In another embodiment, the invention features a method of modulating the expression of more than one BCR-ABL and/or ERG gene in an organism comprising: (a) synthesizing  
5 siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the BCR-ABL and/or ERG gene; and (b) introducing the siNA molecules into the organism under conditions suitable to modulate the expression of the BCR-ABL and/or ERG genes in the organism.

10 In one embodiment, the invention features a method of modulating the expression of a BCR-ABL and/or ERG gene in an organism comprising contacting the organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the BCR-ABL and/or ERG gene in the organism.

In another embodiment, the invention features a method of modulating the expression  
15 of more than one BCR-ABL and/or ERG gene in an organism comprising contacting the organism with one or more siNA molecules of the invention under conditions suitable to modulate the expression of the BCR-ABL and/or ERG genes in the organism.

The siNA molecules of the invention can be designed to inhibit target (BCR-ABL and/or ERG) gene expression through RNAi targeting of a variety of RNA molecules. In one  
20 embodiment, the siNA molecules of the invention are used to target various RNAs corresponding to a target gene. Non-limiting examples of such RNAs include messenger RNA (mRNA), alternate RNA splice variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates. If alternate splicing produces a family of transcripts that are distinguished by usage of  
25 appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically inhibit or to distinguish among the functions of gene family members. For example, a protein that contains an alternatively spliced transmembrane domain can be expressed in both membrane bound and secreted forms. Use of the invention

to target the exon containing the transmembrane domain can be used to determine the functional consequences of pharmaceutical targeting of membrane bound as opposed to the secreted form of the protein. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, pharmaceutical discovery applications, molecular diagnostic and gene function applications, and gene mapping, for example using single nucleotide polymorphism mapping with siNA molecules of the invention. Such applications can be implemented using known gene sequences or from partial sequences available from an expressed sequence tag (EST).

In another embodiment, the siNA molecules of the invention are used to target conserved sequences corresponding to a gene family or gene families such as BCR-ABL and/or ERG family genes. As such, siNA molecules targeting multiple BCR-ABL and/or ERG targets can provide increased therapeutic effect. In addition, siNA can be used to characterize pathways of gene function in a variety of applications. For example, the present invention can be used to inhibit the activity of target gene(s) in a pathway to determine the function of uncharacterized gene(s) in gene function analysis, mRNA function analysis, or translational analysis. The invention can be used to determine potential target gene pathways involved in various diseases and conditions toward pharmaceutical development. The invention can be used to understand pathways of gene expression involved in, for example, the progression and/or maintenance of cancer.

In one embodiment, siNA molecule(s) and/or methods of the invention are used to inhibit the expression of gene(s) that encode RNA referred to by Genbank Accession, for example BCR-ABL and/or ERG genes encoding RNA sequence(s) referred to herein by Genbank Accession number, for example Genbank Accession Nos. shown in Table I.

In one embodiment, the invention features a method comprising: (a) generating a library of siNA constructs having a predetermined complexity; and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target RNA sequence. In another embodiment, the siNA molecules of (a) have strands of a fixed length, for example, about 23 nucleotides in length. In yet another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 19 to

about 25 (*e.g.*, about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In one embodiment, the invention features a method comprising: (a) generating a randomized library of siNA constructs having a predetermined complexity, such as of  $4^N$ , where N represents the number of base paired nucleotides in each of the siNA construct strands (*eg.* for a siNA construct having 21 nucleotide sense and antisense strands with 19 base pairs, the complexity would be  $4^{19}$ ); and (b) assaying the siNA constructs of (a) above, under conditions suitable to determine RNAi target sites within the target BCR-ABL and/or ERG RNA sequence. In another embodiment, the siNA molecules of (a) have strands of a fixed length, for example about 23 nucleotides in length. In yet another embodiment, the siNA molecules of (a) are of differing length, for example having strands of about 19 to about 25 (*e.g.*, about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described in Example 7 herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. In another embodiment, fragments of BCR-ABL and/or ERG RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target BCR-ABL and/or ERG RNA sequence. The target BCR-ABL and/or ERG RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In another embodiment, the invention features a method comprising: (a) analyzing the sequence of a RNA target encoded by a target gene; (b) synthesizing one or more sets of siNA molecules having sequence complementary to one or more regions of the RNA of (a);



and (c) assaying the siNA molecules of (b) under conditions suitable to determine RNAi targets within the target RNA sequence. In one embodiment, the siNA molecules of (b) have strands of a fixed length, for example about 23 nucleotides in length. In another embodiment, the siNA molecules of (b) are of differing length, for example having strands of about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. Fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by expression in *in vivo* systems.

By "target site" is meant a sequence within a target RNA that is "targeted" for cleavage mediated by a siNA construct which contains sequences within its antisense region that are complementary to the target sequence.

By "detectable level of cleavage" is meant cleavage of target RNA (and formation of cleaved product RNAs) to an extent sufficient to discern cleavage products above the background of RNAs produced by random degradation of the target RNA. Production of cleavage products from 1-5% of the target RNA is sufficient to detect above the background for most methods of detection.

In one embodiment, the invention features a composition comprising a siNA molecule of the invention, which can be chemically-modified, in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a pharmaceutical composition comprising siNA molecules of the invention, which can be chemically-modified, targeting one or more genes in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a method for treating or preventing a disease or condition in a subject, comprising administering to the subject a composition of the invention under conditions suitable for the treatment or prevention of the disease or condition in the subject, alone or in conjunction with one or more other therapeutic compounds. In yet another

embodiment, the invention features a method for reducing or preventing tissue rejection in a subject comprising administering to the subject a composition of the invention under conditions suitable for the reduction or prevention of tissue rejection in the subject.

In another embodiment, the invention features a method for validating a BCR-ABL and/or ERG gene target, comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a BCR-ABL and/or ERG target gene; (b) introducing the siNA molecule into a cell, tissue, or organism under conditions suitable for modulating expression of the BCR-ABL and/or ERG target gene in the cell, tissue, or organism; and (c) determining the function of the gene by assaying for any phenotypic change in the cell, tissue, or organism.

In another embodiment, the invention features a method for validating a BCR-ABL and/or ERG target comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a BCR-ABL and/or ERG target gene; (b) introducing the siNA molecule into a biological system under conditions suitable for modulating expression of the BCR-ABL and/or ERG target gene in the biological system; and (c) determining the function of the gene by assaying for any phenotypic change in the biological system.

By "biological system" is meant, material, in a purified or unpurified form, from biological sources, including but not limited to human, animal, plant, insect, bacterial, viral or other sources, wherein the system comprises the components required for RNAi activity. The term "biological system" includes, for example, a cell, tissue, or organism, or extract thereof. The term biological system also includes reconstituted RNAi systems that can be used in an *in vitro* setting.

By "phenotypic change" is meant any detectable change to a cell that occurs in response to contact or treatment with a nucleic acid molecule of the invention (e.g., siNA). Such detectable changes include, but are not limited to, changes in shape, size, proliferation, motility, protein expression or RNA expression or other physical or chemical changes as can

be assayed by methods known in the art. The detectable change can also include expression of reporter genes/molecules such as Green Florescent Protein (GFP) or various tags that are used to identify an expressed protein or any other cellular component that can be assayed.

5 In one embodiment, the invention features a kit containing a siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of a BCR-ABL and/or ERG target gene in a cell, tissue, or organism. In another embodiment, the invention features a kit containing more than one siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of more than one BCR-ABL and/or ERG target gene in a cell, tissue, or organism.

10 In one embodiment, the invention features a kit containing a siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of a BCR-ABL and/or ERG target gene in a biological system. In another embodiment, the invention features a kit containing more than one siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of more than one BCR-  
15 ABL and/or ERG target gene in a biological system.

In one embodiment, the invention features a cell containing one or more siNA molecules of the invention, which can be chemically-modified. In another embodiment, the cell containing a siNA molecule of the invention is a mammalian cell. In yet another embodiment, the cell containing a siNA molecule of the invention is a human cell.

20 In one embodiment, the synthesis of a siNA molecule of the invention, which can be chemically-modified, comprises: (a) synthesis of two complementary strands of the siNA molecule; (b) annealing the two complementary strands together under conditions suitable to obtain a double-stranded siNA molecule. In another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase oligonucleotide synthesis. In  
25 yet another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase tandem oligonucleotide synthesis.

In one embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing a first oligonucleotide sequence strand of the siNA

molecule, wherein the first oligonucleotide sequence strand comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of the second oligonucleotide sequence strand of the siNA; (b) synthesizing the second oligonucleotide sequence strand of siNA on the scaffold of the first oligonucleotide sequence strand, wherein the second  
5 oligonucleotide sequence strand further comprises a chemical moiety than can be used to purify the siNA duplex; (c) cleaving the linker molecule of (a) under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex; and (d) purifying the siNA duplex utilizing the chemical moiety of the second oligonucleotide sequence strand. In one embodiment, cleavage of the linker molecule in (c) above takes place during  
10 deprotection of the oligonucleotide, for example under hydrolysis conditions using an alkylamine base such as methylamine. In one embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a  
15 scaffold for synthesizing the second strand can comprise similar reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place concomitantly. In another embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group, which can be employed in a trityl-on  
20 synthesis strategy as described herein. In yet another embodiment, the chemical moiety, such as a dimethoxytrityl group, is removed during purification, for example, using acidic conditions.

In a further embodiment, the method for siNA synthesis is a solution phase synthesis or hybrid phase synthesis wherein both strands of the siNA duplex are synthesized in tandem  
25 using a cleavable linker attached to the first sequence which acts a scaffold for synthesis of the second sequence. Cleavage of the linker under conditions suitable for hybridization of the separate siNA sequence strands results in formation of the double-stranded siNA molecule.

In another embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing one oligonucleotide sequence strand of the siNA molecule, wherein the sequence comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of another oligonucleotide sequence; (b) synthesizing a second  
5 oligonucleotide sequence having complementarity to the first sequence strand on the scaffold of (a), wherein the second sequence comprises the other strand of the double-stranded siNA molecule and wherein the second sequence further comprises a chemical moiety that can be used to isolate the attached oligonucleotide sequence; (c) purifying the product of (b) utilizing the chemical moiety of the second oligonucleotide sequence strand under conditions  
10 suitable for isolating the full-length sequence comprising both siNA oligonucleotide strands connected by the cleavable linker and under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example under hydrolysis conditions. In another embodiment, cleavage of the linker  
15 molecule in (c) above takes place after deprotection of the oligonucleotide. In another embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can  
20 comprise similar reactivity or differing reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place either concomitantly or sequentially. In one embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group.

25 In another embodiment, the invention features a method for making a double-stranded siNA molecule in a single synthetic process comprising: (a) synthesizing an oligonucleotide having a first and a second sequence, wherein the first sequence is complementary to the second sequence, and the first oligonucleotide sequence is linked to the second sequence via a cleavable linker, and wherein a terminal 5'-protecting group, for example, a 5'-O-  
30 dimethoxytrityl group (5'-O-DMT) remains on the oligonucleotide having the second

sequence; (b) deprotecting the oligonucleotide whereby the deprotection results in the cleavage of the linker joining the two oligonucleotide sequences; and (c) purifying the product of (b) under conditions suitable for isolating the double-stranded siNA molecule, for example using a trityl-on synthesis strategy as described herein.

5        In another embodiment, the method of synthesis of siNA molecules of the invention comprises the teachings of Scaringe *et al.*, US Patent Nos. 5,889,136; 6,008,400; and 6,111,086, incorporated by reference herein in their entirety.

10        In one embodiment, the invention features siNA constructs that mediate RNAi against a BCR-ABL and/or ERG, wherein the siNA construct comprises one or more chemical modifications, for example, one or more chemical modifications having any of Formulae I-VII or any combination thereof that increases the nuclease resistance of the siNA construct.

15        In another embodiment, the invention features a method for generating siNA molecules with increased nuclease resistance comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased nuclease resistance.

20        In one embodiment, the invention features siNA constructs that mediate RNAi against a BCR-ABL and/or ERG, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the sense and antisense strands of the siNA construct.

25        In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the sense and antisense strands of the siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the sense and antisense strands of the siNA molecule.

In one embodiment, the invention features siNA constructs that mediate RNAi against a BCR-ABL and/or ERG, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target RNA sequence within a cell.

5 In one embodiment, the invention features siNA constructs that mediate RNAi against a BCR-ABL and/or ERG, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target DNA sequence within a cell.

10 In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence.

15 In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence.

20 In one embodiment, the invention features siNA constructs that mediate RNAi against a BCR-ABL and/or ERG, wherein the siNA construct comprises one or more chemical modifications described herein that modulate the polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA construct.

In another embodiment, the invention features a method for generating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to a chemically-modified siNA molecule comprising (a) introducing nucleotides having any of  
5 Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the chemically-modified siNA molecule.

10 In one embodiment, the invention features chemically-modified siNA constructs that mediate RNAi against a BCR-ABL and/or ERG in a cell, wherein the chemical modifications do not significantly effect the interaction of siNA with a target RNA molecule, DNA molecule and/or proteins or other factors that are essential for RNAi in a manner that would decrease the efficacy of RNAi mediated by such siNA constructs.

15 In another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against BCR-ABL and/or ERG comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity.

20 In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against a BCR-ABL and/or ERG target RNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the  
25 target RNA.

In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against a BCR-ABL and/or ERG target DNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination



thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target DNA.

5 In one embodiment, the invention features siNA constructs that mediate RNAi against a BCR-ABL and/or ERG, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the cellular uptake of the siNA construct.

10 In another embodiment, the invention features a method for generating siNA molecules against BCR-ABL and/or ERG with improved cellular uptake comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved cellular uptake.

15 In one embodiment, the invention features siNA constructs that mediate RNAi against a BCR-ABL and/or ERG, wherein the siNA construct comprises one or more chemical modifications described herein that increases the bioavailability of the siNA construct, for example, by attaching polymeric conjugates such as polyethyleneglycol or equivalent conjugates that improve the pharmacokinetics of the siNA construct, or by attaching conjugates that target specific tissue types or cell types *in vivo*. Non-limiting examples of such conjugates are described in Vargeese *et al.*, U.S. Serial No. 10/201,394 incorporated by reference herein.

20 In one embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability, comprising (a) introducing a conjugate into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such conjugates can include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code  
25 sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; polyamines, such as spermine or spermidine; and others.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing an excipient formulation to a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such  
5 excipients include polymers such as cyclodextrins, lipids, cationic lipids, polyamines, phospholipids, and others.

In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing nucleotides having any of Formulae I-VII or any combination thereof into a siNA molecule, and (b) assaying the  
10 siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability.

In another embodiment, polyethylene glycol (PEG) can be covalently attached to siNA compounds of the present invention. The attached PEG can be any molecular weight, preferably from about 2,000 to about 50,000 daltons (Da).

15 The present invention can be used alone or as a component of a kit having at least one of the reagents necessary to carry out the *in vitro* or *in vivo* introduction of RNA to test samples and/or subjects. For example, preferred components of the kit include a siNA molecule of the invention and a vehicle that promotes introduction of the siNA into cells of interest as described herein (e.g., using lipids and other methods of transfection known in the  
20 art, see for example Beigelman *et al*, US 6,395,713). The kit can be used for target validation, such as in determining gene function and/or activity, or in drug optimization, and in drug discovery (see for example Usman *et al.*, USSN 60/402,996). Such a kit can also include instructions to allow a user of the kit to practice the invention.

The term "short interfering nucleic acid", "siNA", "short interfering RNA", "siRNA",  
25 "short interfering nucleic acid molecule", "short interfering oligonucleotide molecule", or "chemically-modified short interfering nucleic acid molecule" as used herein refers to any nucleic acid molecule capable of inhibiting or down regulating gene expression or viral replication, for example by mediating RNA interference "RNAi" or gene silencing in a

sequence-specific manner; see for example Bass, 2001, *Nature*, 411, 428-429; Elbashir *et al.*, 2001, *Nature*, 411, 494-498; and Kreutzer *et al.*, International PCT Publication No. WO 00/44895; Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck *et al.*, International PCT  
5 Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.*, International PCT Publication No. WO 00/44914; Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237; Hutvagner and Zamore, 2002, *Science*,  
10 297, 2056-60; McManus *et al.*, 2002, *RNA*, 8, 842-850; Reinhart *et al.*, 2002, *Gene & Dev.*, 16, 1616-1626; and Reinhart & Bartel, 2002, *Science*, 297, 1831). Non limiting examples of siNA molecules of the invention are shown in Figures 4-6, and Tables II, III, and IV herein. For example the siNA can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises  
15 nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each  
20 strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for example wherein the double stranded region is about 19 base pairs); the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises  
25 nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. Alternatively, the siNA is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s). The siNA can be a polynucleotide with a hairpin secondary structure, having self-complementary sense and antisense regions, wherein the  
30 antisense region comprises nucleotide sequence that is complementary to nucleotide

sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein

5 the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active siNA molecule capable of mediating RNAi. The siNA can also comprise a single

10 stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such siNA molecule does not require the presence within the siNA molecule of nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide can further comprise a terminal phosphate group, such as a 5'-

15 phosphate (see for example Martinez *et al.*, 2002, *Cell.*, 110, 563-574 and Schwarz *et al.*, 2002, *Molecular Cell*, 10, 537-568), or 5',3'-diphosphate. In certain embodiment, the siNA molecule of the invention comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linker molecules as is known in the art, or are alternately non-covalently linked

20 by ionic interactions, hydrogen bonding, van der Waals interactions, hydrophobic interactions, and/or stacking interactions. In certain embodiments, the siNA molecules of the invention comprise nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the siNA molecule of the invention interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the

25 target gene. As used herein, siNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules of the invention lack 2'-hydroxy (2'-OH) containing nucleotides. Applicant describes in certain embodiments short interfering nucleic acids that do not require the presence of nucleotides

30 having a 2'-hydroxy group for mediating RNAi and as such, short interfering nucleic acid

molecules of the invention optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such siNA molecules that do not require the presence of ribonucleotides within the siNA molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, siNA molecules can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. The modified short interfering nucleic acid molecules of the invention can also be referred to as short interfering modified oligonucleotides "siMON." As used herein, the term siNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically-modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, or epigenetics. For example, siNA molecules of the invention can be used to epigenetically silence genes at both the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by siNA molecules of the invention can result from siNA mediated modification of chromatin structure to alter gene expression (see, for example, Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237).

By "modulate" is meant that the expression of the gene, or level of RNA molecule or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the absence of the modulator. For example, the term "modulate" can mean "inhibit," but the use of the word "modulate" is not limited to this definition.

By "inhibit", "down-regulate", or "reduce", it is meant that the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, is reduced below that observed in the absence of the nucleic acid molecules (e.g., siNA) of the invention. In one embodiment, inhibition, down-regulation or reduction with an siNA molecule is below that level observed in the presence of an inactive or attenuated molecule. In another embodiment, inhibition, down-regulation, or reduction with siNA molecules is below that level observed in the presence of, for example, an siNA molecule with scrambled sequence or with mismatches. In another embodiment, inhibition, down-regulation, or reduction of gene expression with a nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its absence.

By "gene" or "target gene" is meant, a nucleic acid that encodes an RNA, for example, nucleic acid sequences including, but not limited to, structural genes encoding a polypeptide. The target gene can be a gene derived from a cell, an endogenous gene, a transgene, or exogenous genes such as genes of a pathogen, for example a virus, which is present in the cell after infection thereof. The cell containing the target gene can be derived from or contained in any organism, for example a plant, animal, protozoan, virus, bacterium, or fungus. Non-limiting examples of plants include monocots, dicots, or gymnosperms. Non-limiting examples of animals include vertebrates or invertebrates. Non-limiting examples of fungi include molds or yeasts.

By "BCR-ABL" is meant, a BCR-ABL polypeptide, protein and/or a polynucleotide encoding a BCR-ABL protein (such as BCR-ABL fusion polynucleotides referred to in Table I or any other BCR-ABL transcript derived from a BCR-ABL fusion gene).

By "BCR-ABL protein" is meant, a BCR-ABL peptide or protein or a component thereof, wherein the peptide or protein is encoded by a BCR-ABL gene.

By "ERG" is meant, a polypeptide or protein comprising an Ets family type transcription factor or fusion variant thereof or polynucleotide encoding an Ets family type

transcription factor or fusion variant thereof (such as ERG fusion polynucleotides referred to in Table I or any other ERG transcript derived from an ERG fusion gene).

By "ERG protein" is meant, a ERG peptide or protein or a component thereof, wherein the peptide or protein is encoded by a ERG or ERG fusion gene.

5 By "cancer" is meant a group of diseases characterized by uncontrolled growth and spread of abnormal cells. In certain embodiments, the term cancer as used herein refers to leukemia, such as chronic myelogenous leukemia (CML) resulting from the BCR-ABL fusion gene.

10 By "highly conserved sequence region" is meant, a nucleotide sequence of one or more regions in a target gene does not vary significantly from one generation to the other or from one biological system to the other.

By "sense region" is meant a nucleotide sequence of a siNA molecule having complementarity to an antisense region of the siNA molecule. In addition, the sense region of a siNA molecule can comprise a nucleic acid sequence having homology with a target  
15 nucleic acid sequence.

By "antisense region" is meant a nucleotide sequence of a siNA molecule having complementarity to a target nucleic acid sequence. In addition, the antisense region of a siNA molecule can optionally comprise a nucleic acid sequence having complementarity to a sense region of the siNA molecule.

20 By "target nucleic acid" is meant any nucleic acid sequence whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA.

By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy  
25 for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. Determination of

binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner *et al.*, 1987, *CSH Symp. Quant. Biol.* LII pp.123-133; Frier *et al.*, 1986, *Proc. Nat. Acad. Sci. USA* 83:9373-9377; Turner *et al.*, 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule  
5 that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence.

10 The siNA molecules of the invention represent a novel therapeutic approach to treat various diseases and conditions, including cancer (e.g. leukemia such as CML) and any other indications that can respond to the level of BCR-ABL in a cell or tissue.

The siRNA molecules of the invention also represent a novel therapeutic approach to treat a treat a broad spectrum of oncology and neovascularization-related indications,  
15 including but not limited to cancers of the lung, colon, breast, prostate, cervix, lymphoma, Ewing's sarcoma and related tumors, melanoma, angiogenic disease states such as tumor angiogenesis, diabetic retinopathy, macular degeneration, neovascular glaucoma, myopic degeneration, arthritis such as rheumatoid arthritis, psoriasis, verruca vulgaris, angiofibroma of tuberous sclerosis, port-wine stains, Sturge Weber syndrome, Kippel-Trenaunay-Weber  
20 syndrome, Osler-Weber-rendu syndrome, leukemias such as acute myeloid leukemia, osteoporosis, wound healing and other indications that can respond to the level of ERG in a cell or tissue.

In one embodiment of the present invention, each sequence of a siNA molecule of the invention is independently about 18 to about 24 nucleotides in length, in specific  
25 embodiments about 18, 19, 20, 21, 22, 23, or 24 nucleotides in length. In another embodiment, the siNA duplexes of the invention independently comprise about 17 to about 23 base pairs (e.g., about 17, 18, 19, 20, 21, 22 or 23). In yet another embodiment, siNA molecules of the invention comprising hairpin or circular structures are about 35 to about 55 (e.g., about 35, 40, 45, 50 or 55) nucleotides in length, or about 38 to about 44 (e.g., 38, 39,



40, 41, 42, 43 or 44) nucleotides in length and comprising about 16 to about 22 (e.g., about 16, 17, 18, 19, 20, 21 or 22) base pairs. Exemplary siNA molecules of the invention are shown in Table II. Exemplary synthetic siNA molecules of the invention are shown in Tables III and IV and/or Figures 4-5.

5 As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell can be present in an organism, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell). The cell can be of somatic or germ line origin,  
10 totipotent or pluripotent, dividing or non-dividing. The cell can also be derived from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

The siNA molecules of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, infusion pump or stent, with or without their incorporation  
15 in biopolymers. In particular embodiments, the nucleic acid molecules of the invention comprise sequences shown in Tables II-III and/or Figures 4-5. Examples of such nucleic acid molecules consist essentially of sequences defined in these tables and figures. Furthermore, the chemically modified constructs described in Table IV can be applied to any  
20 siNA sequence of the invention.

In another aspect, the invention provides mammalian cells containing one or more siNA molecules of this invention. The one or more siNA molecules can independently be targeted to the same or different sites.

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By  
25 "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a  $\beta$ -D-ribofuranose moiety. The terms include double-stranded RNA, single-stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the

addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in the RNA molecules of the instant invention can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

By "subject" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Subject" also refers to an organism to which the nucleic acid molecules of the invention can be administered. In one embodiment, a subject is a mammal or mammalian cells. In another embodiment, a subject is a human or human cells.

The term "phosphorothioate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise a sulfur atom. Hence, the term phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

The term "universal base" as used herein refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art (see for example Loakes, 2001, *Nucleic Acids Research*, 29, 2437-2447).

The term "acyclic nucleotide" as used herein refers to any nucleotide having an acyclic ribose sugar, for example where any of the ribose carbons (C1, C2, C3, C4, or C5), are independently or in combination absent from the nucleotide.

The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions discussed herein (e.g., cancers and othe proliferative conditions). For example, to treat a particular disease or condition, the siNA molecules can be administered to a subject or can be administered to other appropriate cells evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

In a further embodiment, the siNA molecules can be used in combination with other known treatments to treat conditions or diseases discussed above. For example, the described molecules could be used in combination with one or more known therapeutic agents to treat a disease or condition. Non-limiting examples of other therapeutic agents that  
5 can be readily combined with a siNA molecule of the invention are enzymatic nucleic acid molecules, allosteric nucleic acid molecules, antisense, decoy, or aptamer nucleic acid molecules, antibodies such as monoclonal antibodies, small molecules, and other organic and/or inorganic compounds including metals, salts and ions.

In one embodiment, the invention features an expression vector comprising a nucleic  
10 acid sequence encoding at least one siNA molecule of the invention, in a manner which allows expression of the siNA molecule. For example, the vector can contain sequence(s) encoding both strands of a siNA molecule comprising a duplex. The vector can also contain sequence(s) encoding a single nucleic acid molecule that is self-complementary and thus forms a siNA molecule. Non-limiting examples of such expression vectors are described in  
15 Paul *et al.*, 2002, *Nature Biotechnology*, 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee *et al.*, 2002, *Nature Biotechnology*, 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725.

In another embodiment, the invention features a mammalian cell, for example, a human cell, including an expression vector of the invention.

20 In yet another embodiment, the expression vector of the invention comprises a sequence for a siNA molecule having complementarity to a RNA molecule referred to by a Genbank Accession numbers, for example Genbank Accession Nos. shown in Table I.

In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more siNA molecules, which can be the same or different.

25 In another aspect of the invention, siNA molecules that interact with target RNA molecules and down-regulate gene encoding target RNA molecules (for example target RNA molecules referred to by Genbank Accession numbers herein) are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA

plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient  
5 expression of siNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siNA molecules bind and down-regulate gene function or expression via RNA interference (RNAi). Delivery of siNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow  
10 for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

## 15 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a non-limiting example of a scheme for the synthesis of siNA molecules. The complementary siNA sequence strands, strand 1 and strand 2, are synthesized in tandem and are connected by a cleavable linkage, such as a nucleotide succinate or abasic succinate, which can be the same or different from the cleavable linker  
20 used for solid phase synthesis on a solid support. The synthesis can be either solid phase or solution phase, in the example shown, the synthesis is a solid phase synthesis. The synthesis is performed such that a protecting group, such as a dimethoxytrityl group, remains intact on the terminal nucleotide of the tandem oligonucleotide. Upon cleavage and deprotection of the oligonucleotide, the two siNA strands spontaneously hybridize to form a siNA duplex,  
25 which allows the purification of the duplex by utilizing the properties of the terminal protecting group, for example by applying a trityl on purification method wherein only duplexes/oligonucleotides with the terminal protecting group are isolated.

Figure 2 shows a MALDI-TOV mass spectrum of a purified siNA duplex synthesized by a method of the invention. The two peaks shown correspond to the predicted mass of the separate siNA sequence strands. This result demonstrates that the siNA duplex generated from tandem synthesis can be purified as a single entity using a simple trityl-on purification methodology.

Figure 3 shows a non-limiting proposed mechanistic representation of target RNA degradation involved in RNAi. Double-stranded RNA (dsRNA), which is generated by RNA-dependent RNA polymerase (RdRP) from foreign single-stranded RNA, for example viral, transposon, or other exogenous RNA, activates the DICER enzyme that in turn generates siNA duplexes. Alternately, synthetic or expressed siNA can be introduced directly into a cell by appropriate means. An active siNA complex forms which recognizes a target RNA, resulting in degradation of the target RNA by the RISC endonuclease complex or in the synthesis of additional RNA by RNA-dependent RNA polymerase (RdRP), which can activate DICER and result in additional siNA molecules, thereby amplifying the RNAi response.

Figure 4A-F shows non-limiting examples of chemically-modified siNA constructs of the present invention. In the figure, N stands for any nucleotide (adenosine, guanosine, cytosine, uridine, or optionally thymidine, for example thymidine can be substituted in the overhanging regions designated by parenthesis (N N)). Various modifications are shown for the sense and antisense strands of the siNA constructs.

**Figure 4A:** The sense strand comprises 21 nucleotides having four phosphorothioate 5'- and 3'-terminal internucleotide linkages, wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and four 5'-terminal phosphorothioate internucleotide linkages and

wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

**Figure 4B:** The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

**Figure 4C:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

**Figure 4D:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine

nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

**Figure 4E:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

**Figure 4F:** The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-deoxy nucleotides except for (N N) nucleotides, which

can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand of constructs A-F comprise sequence complementary to any target nucleic acid sequence of the invention.

Figure 5A-F shows non-limiting examples of specific chemically-modified siNA sequences of the invention. A-F applies the chemical modifications described in Figure 4A-F to a BCR-ABL siNA sequence.

Figure 6 shows non-limiting examples of different siNA constructs of the invention. The examples shown (constructs 1, 2, and 3) have 19 representative base pairs; however, different embodiments of the invention include any number of base pairs described herein. Bracketed regions represent nucleotide overhangs, for example comprising about 1, 2, 3, or 4 nucleotides in length, preferably about 2 nucleotides. Constructs 1 and 2 can be used independently for RNAi activity. Construct 2 can comprise a polynucleotide or non-nucleotide linker, which can optionally be designed as a biodegradable linker. In one embodiment, the loop structure shown in construct 2 can comprise a biodegradable linker that results in the formation of construct 1 *in vivo* and/or *in vitro*. In another example, construct 3 can be used to generate construct 2 under the same principle wherein a linker is used to generate the active siNA construct 2 *in vivo* and/or *in vitro*, which can optionally utilize another biodegradable linker to generate the active siNA construct 1 *in vivo* and/or *in vitro*. As such, the stability and/or activity of the siNA constructs can be modulated based on the design of the siNA construct for use *in vivo* or *in vitro* and/or *in vitro*.

Figure 7A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate siNA hairpin constructs.

Figure 7A: A DNA oligomer is synthesized with a 5'-restriction site (R1) sequence followed by a region having sequence identical (sense region of siNA) to a predetermined BCR-ABL target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, which is followed by a loop sequence of defined sequence (X), comprising, for example, about 3 to about 10 nucleotides.



**Figure 7B:** The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence that will result in a siNA transcript having specificity for a BCR-ABL target sequence and having self-complementary sense and antisense regions.

5       **Figure 7C:** The construct is heated (for example to about 95°C) to linearize the sequence, thus allowing extension of a complementary second DNA strand using a primer to the 3'-restriction sequence of the first strand. The double-stranded DNA is then inserted into an appropriate vector for expression in cells. The construct can be designed such that a 3'-terminal nucleotide overhang results from the transcription, for example by engineering  
10       restriction sites and/or utilizing a poly-U termination region as described in Paul *et al.*, 2002, *Nature Biotechnology*, 29, 505-508.

**Figure 8A-C** is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate double-stranded siNA constructs.

15       **Figure 8A:** A DNA oligomer is synthesized with a 5'-restriction (R1) site sequence followed by a region having sequence identical (sense region of siNA) to a predetermined BCR-ABL target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, and which is followed by a 3'-restriction site (R2) which is adjacent to a loop sequence of defined sequence (X).

20       **Figure 8B:** The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence.

25       **Figure 8C:** The construct is processed by restriction enzymes specific to R1 and R2 to generate a double-stranded DNA which is then inserted into an appropriate vector for expression in cells. The transcription cassette is designed such that a U6 promoter region flanks each side of the dsDNA which generates the separate sense and antisense strands of the siNA. Poly T termination sequences can be added to the constructs to generate U overhangs in the resulting transcript.

Figure 9A-E is a diagrammatic representation of a method used to determine target sites for siNA mediated RNAi within a particular target nucleic acid sequence, such as messenger RNA.

5      **Figure 9A:** A pool of siNA oligonucleotides are synthesized wherein the antisense region of the siNA constructs has complementarity to target sites across the target nucleic acid sequence, and wherein the sense region comprises sequence complementary to the antisense region of the siNA.

**Figure 9B&C:** (**Figure 9B**) The sequences are pooled and are inserted into vectors such that (**Figure 9C**) transfection of a vector into cells results in the expression of the siNA.

10      **Figure 9D:** Cells are sorted based on phenotypic change that is associated with modulation of the target nucleic acid sequence.

**Figure 9E:** The siNA is isolated from the sorted cells and is sequenced to identify efficacious target sites within the target nucleic acid sequence.

**Figure 10** shows non-limiting examples of different stabilization chemistries (1-10) that can be used, for example, to stabilize the 3'-end of siNA sequences of the invention, including (1) [3-3']-inverted deoxyribose; (2) deoxyribonucleotide; (3) [5'-3']-3'-deoxyribonucleotide; (4) [5'-3']-ribonucleotide; (5) [5'-3']-3'-O-methyl ribonucleotide; (6) 3'-glyceryl; (7) [3'-5']-3'-deoxyribonucleotide; (8) [3'-3']-deoxyribonucleotide; (9) [5'-2']-deoxyribonucleotide; and (10) [5-3']-dideoxyribonucleotide. In addition to modified and unmodified backbone chemistries indicated in the figure, these chemistries can be combined with different backbone modifications as described herein, for example, backbone modifications having Formula I. In addition, the 2'-deoxy nucleotide shown 5' to the terminal modifications shown can be another modified or unmodified nucleotide or non-nucleotide described herein, for example modifications having any of Formulae I-VII or any combination thereof.

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**Figure 11** shows a non-limiting example of a strategy used to identify chemically modified siNA constructs of the invention that are nuclease resistance while preserving the

ability to mediate RNAi activity. Chemical modifications are introduced into the siNA construct based on educated design parameters (e.g. introducing 2'-modifications, base modifications, backbone modifications, terminal cap modifications etc). The modified construct is tested in an appropriate system (e.g. human serum for nuclease resistance, shown, or an animal model for PK/delivery parameters). In parallel, the siNA construct is tested for RNAi activity, for example in a cell culture system such as a luciferase reporter assay). Lead siNA constructs are then identified which possess a particular characteristic while maintaining RNAi activity, and can be further modified and assayed once again. This same approach can be used to identify siNA-conjugate molecules with improved pharmacokinetic profiles, delivery, and RNAi activity.

Figure 12A-F shows non-limiting examples of specific chemically-modified siNA sequences of the invention. A-F applies the chemical modifications described in Figure 4A-F to a ERG siNA sequence.

Figure 13 shows a non-limiting example of reduction of ERG2 mRNA in DLD1 cells mediated by siNAs that target ERG2 mRNA. DLD1 cells were transfected with 0.25 ug/well of lipid complexed with 25 nM siNA. A screen of siNA constructs comprising ribonucleotides and 3'-terminal dithymidine caps was compared to untreated cells, scrambled siNA control constructs (Scram1 and Scram2), and cells transfected with lipid alone (transfection control). As shown in the figure, all of the siNA constructs significantly reduce ERG2 RNA expression.

## DETAILED DESCRIPTION OF THE INVENTION

### Mechanism of action of Nucleic Acid Molecules of the Invention

The discussion that follows discusses the proposed mechanism of RNA interference mediated by short interfering RNA as is presently known, and is not meant to be limiting and is not an admission of prior art. Applicant demonstrates herein that chemically-modified short interfering nucleic acids possess similar or improved capacity to mediate RNAi as do siRNA molecules and are expected to possess improved stability and activity *in vivo*; therefore, this discussion is not meant to be limiting only to siRNA and can be applied to

siNA as a whole. By "improved capacity to mediate RNAi" or "improved RNAi activity" is meant to include RNAi activity measured *in vitro* and/or *in vivo* where the RNAi activity is a reflection of both the ability of the siNA to mediate RNAi and the stability of the siNAs of the invention. In this invention, the product of these activities can be increased *in vitro* and/or *in vivo* compared to an all RNA siRNA or a siNA containing a plurality of ribonucleotides. In some cases, the activity or stability of the siNA molecule can be decreased (i.e., less than ten-fold), but the overall activity of the siNA molecule is enhanced *in vitro* and/or *in vivo*.

RNA interference refers to the process of sequence specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.*, 1998, *Nature*, 391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2', 5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as Dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Berstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from Dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes. Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs)

from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having  
5 sequence homologous to the siRNA. Cleavage of the target RNA takes place in the middle of the region complementary to the guide sequence of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). In addition, RNA interference can also involve small RNA (e.g., micro-RNA or miRNA) mediated gene silencing, presumably through cellular mechanisms that regulate chromatin structure and thereby prevent transcription of target gene sequences  
10 (see for example Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237). As such, siRNA molecules of the invention can be used to mediate gene silencing via interaction with RNA transcripts or alternately by interaction with particular gene sequences, wherein such interaction results in gene silencing either at the transcriptional level  
15 or post-transcriptional level.

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond *et al.*, 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001,  
20 *Nature*, 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21 nucleotide siRNA duplexes are  
25 most active when containing two 2-nucleotide 3'-terminal nucleotide overhangs. Furthermore, substitution of one or both siRNA strands with 2'-deoxy or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of 3'-terminal siRNA nucleotides with deoxy nucleotides was shown to be tolerated. Mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also  
30 indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the

siRNA guide sequence rather than the 3'-end (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell*, 107, 309); however, siRNA molecules lacking a 5'-phosphate are active when introduced exogenously, suggesting that 5'-phosphorylation of siRNA constructs may occur *in vivo*.

#### Synthesis of Nucleic acid Molecules

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small" refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, individual siNA oligonucleotide sequences or siNA sequences synthesized in tandem) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of protein and/or RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (*e.g.*, certain modified oligonucleotides or portions of oligonucleotides lacking ribonucleotides) are synthesized using protocols known in the art, for example as described in Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19, Thompson *et al.*, International PCT Publication No. WO 99/54459, Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, Brennan *et al.*, 1998, *Biotechnol Bioeng.*, 61, 33-45, and Brennan, U.S. Pat. No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2  $\mu$ mol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 sec coupling step for 2'-deoxy nucleotides or 2'-deoxy-2'-fluoro nucleotides. Table V outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively,

syntheses at the 0.2  $\mu\text{mol}$  scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60  $\mu\text{L}$  of 0.11 M = 6.6  $\mu\text{mol}$ ) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60  $\mu\text{L}$  of 0.25 M = 15  $\mu\text{mol}$ ) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40  $\mu\text{L}$  of 0.11 M = 4.4  $\mu\text{mol}$ ) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40  $\mu\text{L}$  of 0.25 M = 10  $\mu\text{mol}$ ) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM  $\text{I}_2$ , 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

Deprotection of the DNA-based oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to -20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H<sub>2</sub>O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The method of synthesis used for RNA including certain siNA molecules of the invention follows the procedure as described in Usman *et al.*, 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.*, 1990, *Nucleic Acids Res.*, 18, 5433; and Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, and makes use of

common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2  $\mu\text{mol}$  scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. Table V outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2  $\mu\text{mol}$  scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60  $\mu\text{L}$  of 0.11 M = 6.6  $\mu\text{mol}$ ) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60  $\mu\text{L}$  of 0.25 M = 15  $\mu\text{mol}$ ) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120  $\mu\text{L}$  of 0.11 M = 13.2  $\mu\text{mol}$ ) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120  $\mu\text{L}$  of 0.25 M = 30  $\mu\text{mol}$ ) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM  $\text{I}_2$ , 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide 0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to -20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H<sub>2</sub>O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the



oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300  $\mu$ L of a solution of 1.5 mL N-methylpyrrolidinone, 750  $\mu$ L TEA and 1 mL TEA $\cdot$ 3HF to provide a 1.4 M HF concentration) and heated to 65  $^{\circ}$ C. After 1.5 h, the oligomer is quenched with 1.5 M  $\text{NH}_4\text{HCO}_3$ .

5        Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65  $^{\circ}$ C for 15 min. The vial is brought to rt. TEA $\cdot$ 3HF (0.1 mL) is added and the vial is heated at 65  $^{\circ}$ C for 15 min. The sample is cooled at  $-20^{\circ}$  C and then quenched with 1.5 M  $\text{NH}_4\text{HCO}_3$ .

10        For purification of the trityl-on oligomers, the quenched  $\text{NH}_4\text{HCO}_3$  solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 min. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30%  
15        acetonitrile.

The average stepwise coupling yields are typically >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96-well format.

20        Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example, by ligation (Moore *et al.*, 1992, *Science* 256, 9923; Draper *et al.*, International PCT publication No. WO 93/23569; Shabarova *et al.*, 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.*, 1997, *Nucleosides & Nucleotides*, 16, 951; Bellon *et al.*, 1997, *Bioconjugate Chem.* 8, 204), or by hybridization  
25        following synthesis and/or deprotection.

The siNA molecules of the invention can also be synthesized via a tandem synthesis methodology as described in Example 1 herein, wherein both siNA strands are synthesized as

a single contiguous oligonucleotide fragment or strand separated by a cleavable linker which is subsequently cleaved to provide separate siNA fragments or strands that hybridize and permit purification of the siNA duplex. The linker can be a polynucleotide linker or a non-nucleotide linker. The tandem synthesis of siNA as described herein can be readily adapted  
5 to both multiwell/multiplate synthesis platforms such as 96 well or similarly larger multi-well platforms. The tandem synthesis of siNA as described herein can also be readily adapted to large scale synthesis platforms employing batch reactors, synthesis columns and the like.

A siNA molecule can also be assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes  
10 the antisense region of the RNA molecule.

The nucleic acid molecules of the present invention can be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163). siNA constructs can be purified by  
15 gel electrophoresis using general methods or can be purified by high pressure liquid chromatography (HPLC; see Wincott *et al.*, *supra*, the totality of which is hereby incorporated herein by reference) and re-suspended in water.

In another aspect of the invention, siNA molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA  
20 plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules.

#### 25 Optimizing Activity of the nucleic acid molecule of the invention.

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) can prevent their degradation by serum ribonucleases, which can increase their potency (see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*,

1990 *Nature* 344, 565; Pieken *et al.*, 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162; Sproat, U.S. Pat. No. 5,334,711; Gold *et al.*, U.S. Pat. No. 6,300,074; and Burgin *et al.*, *supra*; all of which are  
5 incorporated by reference herein). All of the above references describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein. Modifications that enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

10 There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-O-  
15 allyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TTBS*, 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.*, 1996, *Biochemistry*, 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein *et al.*, International Publication PCT No. WO 92/07065; Perrault *et al.*, *Nature*, 1990, 344, 565-568; Pieken *et al.*, *Science*, 1991, 253, 314-  
20 317; Usman and Cedergren, *Trends in Biochem. Sci.*, 1992, 17, 334-339; Usman *et al.*, International Publication PCT No. WO 93/15187; Sproat, U.S. Pat. No. 5,334,711 and Beigelman *et al.*, 1995, *J. Biol. Chem.*, 270, 25702; Beigelman *et al.*, International PCT publication No. WO 97/26270; Beigelman *et al.*, U.S. Pat. No. 5,716,824; Usman *et al.*, U.S. Pat. No. 5,627,053; Woolf *et al.*, International PCT Publication No. WO 98/13526;  
25 Thompson *et al.*, USSN 60/082,404 which was filed on April 20, 1998; Karpeisky *et al.*, 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic Acid Sciences)*, 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.*, 67, 99-134; and Burlina *et al.*, 1997, *Bioorg. Med. Chem.*, 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general  
30 methods and strategies to determine the location of incorporation of sugar, base and/or

phosphate modifications and the like into nucleic acid molecules without modulating catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the siNA nucleic acid molecules of the instant invention so long as the ability of siNA to promote RNAi in cells is not significantly inhibited.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorodithioate, and/or 5'-methylphosphonate linkages improves stability, excessive modifications can cause some toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages should be minimized. The reduction in the concentration of these linkages should lower toxicity, resulting in increased efficacy and higher specificity of these molecules.

Short interfering nucleic acid (siNA) molecules having chemical modifications that maintain or enhance activity are provided. Such a nucleic acid is also generally more resistant to nucleases than an unmodified nucleic acid. Accordingly, the *in vitro* and/or *in vivo* activity should not be significantly lowered. In cases in which modulation is the goal, therapeutic nucleic acid molecules delivered exogenously should optimally be stable within cells until translation of the target RNA has been modulated long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677; Caruthers *et al.*, 1992, *Methods in Enzymology* 211,3-19 (incorporated by reference herein)) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability, as described above.

In one embodiment, nucleic acid molecules of the invention include one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.*, 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical

thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets, complementary sequences, or template strands. In another embodiment, nucleic acid  
5 molecules of the invention include one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) LNA "locked nucleic acid" nucleotides such as a 2', 4'-C methylene bicyclo nucleotide (see for example Wengel *et al.*, International PCT Publication No. WO 00/66604 and WO 99/14226).

In another embodiment, the invention features conjugates and/or complexes of siNA  
10 molecules of the invention. Such conjugates and/or complexes can be used to facilitate delivery of siNA molecules into a biological system, such as a cell. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention  
15 encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including, but not limited to, small molecules, lipids, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed  
20 to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid molecules of the invention into a number of cell types originating from different tissues, in the presence or absence of serum (see Sullenger and Cech, U.S. Pat. No. 5,854,038). Conjugates of the molecules described herein can be attached to biologically  
25 active molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

The term "biodegradable linker" as used herein, refers to a nucleic acid or non-nucleic acid linker molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule to a siNA molecule of the

invention or the sense and antisense strands of a siNA molecule of the invention. The biodegradable linker is designed such that its stability can be modulated for a particular purpose, such as delivery to a particular tissue or cell type. The stability of a nucleic acid-based biodegradable linker molecule can be modulated by using various chemistries, for example combinations of ribonucleotides, deoxyribonucleotides, and chemically-modified nucleotides, such as 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus-based linkage, for example, a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

The term "biodegradable" as used herein, refers to degradation in a biological system, for example enzymatic degradation or chemical degradation.

The term "biologically active molecule" as used herein, refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system. Non-limiting examples of biologically active siNA molecules either alone or in combination with other molecules contemplated by the instant invention include therapeutically active molecules such as antibodies, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus-

containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

Therapeutic nucleic acid molecules (e.g., siNA molecules) delivered exogenously optimally are stable within cells until reverse transcription of the RNA has been modulated  
5 long enough to reduce the levels of the RNA transcript. The nucleic acid molecules are resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

10 In yet another embodiment, siNA molecules having chemical modifications that maintain or enhance enzymatic activity of proteins involved in RNAi are provided. Such nucleic acids are also generally more resistant to nucleases than unmodified nucleic acids. Thus, *in vitro* and/or *in vivo* the activity should not be significantly lowered.

Use of the nucleic acid-based molecules of the invention will lead to better treatment of  
15 the disease progression by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes; nucleic acid molecules coupled with known small molecule modulators; or intermittent treatment with combinations of molecules, including different motifs and/or other chemical or biological molecules). The treatment of subjects with siNA molecules can also include combinations of different types of nucleic  
20 acid molecules, such as enzymatic nucleic acid molecules (ribozymes), allozymes, antisense, 2,5-A oligoadenylate, decoys, and aptamers.

In another aspect a siNA molecule of the invention comprises one or more 5' and/or a 3'- cap structure, for example on only the sense siNA strand, the antisense siNA strand, or both siNA strands.

25 By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Adamic *et al.*, U.S. Pat. No. 5,998,203, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or

localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'-terminal (3'-cap) or may be present on both termini. In non-limiting examples, the 5'-cap is selected from the group consisting of glyceryl, inverted deoxy abasic residue (moiety); 4',5'-methylene nucleotide; 1-(beta-D-erythrofuransyl) nucleotide, 4'-thio nucleotide; carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuransyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety.

In non-limiting examples, the 3'-cap is selected from the group consisting of glyceryl, inverted deoxy abasic residue (moiety), 4',5'-methylene nucleotide; 1-(beta-D-erythrofuransyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate; 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuransyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly



recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine and therefore lacks a base at the 1'-position.

An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO<sub>2</sub> or N(CH<sub>3</sub>)<sub>2</sub>, amino, or SH. The term also includes alkenyl groups that are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably, it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO<sub>2</sub>, halogen, N(CH<sub>3</sub>)<sub>2</sub>, amino, or SH. The term "alkyl" also includes alkynyl groups that have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably, it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO<sub>2</sub> or N(CH<sub>3</sub>)<sub>2</sub>, amino or SH.

Such alkyl groups can also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group that has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms.

Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, *supra*; Eckstein *et al.*, International PCT Publication No. WO 92/07065; Usman *et al.*, International PCT Publication No. WO 93/15187; Uhlman & Peyman, *supra*, all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.*, 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (*e.g.*, 5-methylcytidine), 5-alkyluridines (*e.g.*, ribothymidine), 5-halouridine (*e.g.*, 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (*e.g.* 6-methyluridine), propyne, and others (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090; Uhlman & Peyman, *supra*). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents.

In one embodiment, the invention features modified siNA molecules, with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, phosphotriester, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications, see

Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties*, in *Modern Synthetic Methods*, VCH, 331-417, and Mesmacker *et al.*, 1994, *Novel Backbone Replacements for Oligonucleotides*, in *Carbohydrate Modifications in Antisense Research*, ACS, 24-39.

5 By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, see for example Adamic *et al.*, U.S. Pat. No. 5,998,203.

By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, or uracil joined to the 1' carbon of  $\beta$ -D-ribo-furanose.

10 By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate. Non-limiting examples of modified nucleotides are shown by Formulae I-VII and/or other modifications described herein.

15 In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH<sub>2</sub> or 2'-O- NH<sub>2</sub>, which can be modified or unmodified. Such modified groups are described, for example, in Eckstein *et al.*, U.S. Pat. No. 5,672,695 and Matulic-Adamic *et al.*, U.S. Pat. No. 6,248,878, which are both incorporated by reference in their entireties.

20 Various modifications to nucleic acid siNA structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life *in vitro*, stability, and ease of introduction of such oligonucleotides to the target site, *e.g.*, to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

#### Administration of Nucleic Acid Molecules

25 A siRNA molecule of the invention can be adapted for use to treat for example cancer and other indications that can respond to the level of BCR-ABL and/or ERG in a cell or tissue, alone or in combination with other therapies. For example, a siNA molecule can

comprise a delivery vehicle, including liposomes, for administration to a subject, carriers and diluents and their salts, and/or can be present in pharmaceutically acceptable formulations. Methods for the delivery of nucleic acid molecules are described in Akhtar *et al.*, 1992, *Trends Cell Bio.*, 2, 139; *Delivery Strategies for Antisense Oligonucleotide Therapeutics*, ed. Akhtar, 1995, Maurer *et al.*, 1999, *Mol. Membr. Biol.*, 16, 129-140; Hofland and Huang, 1999, *Handb. Exp. Pharmacol.*, 137, 165-192; and Lee *et al.*, 2000, *ACS Symp. Ser.*, 752, 184-192, all of which are incorporated herein by reference. Beigelman *et al.*, U.S. Pat. No. 6,395,713 and Sullivan *et al.*, PCT WO 94/02595 further describe the general methods for delivery of nucleic acid molecules. These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins (see for example Gonzalez *et al.*, 1999, *Bioconjugate Chem.*, 10, 1068-1074), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722). Alternatively, the nucleic acid/vehicle combination is locally delivered by direct injection or by use of an infusion pump. Direct injection of the nucleic acid molecules of the invention, whether subcutaneous, intramuscular, or intradermal, can take place using standard needle and syringe methodologies, or by needle-free technologies such as those described in Conry *et al.*, 1999, *Clin. Cancer Res.*, 5, 2330-2337 and Barry *et al.*, International PCT Publication No. WO 99/31262. The molecules of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents prevent, modulate the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a subject.

Thus, the invention features a pharmaceutical composition comprising one or more nucleic acid(s) of the invention in an acceptable carrier, such as a stabilizer, buffer, and the like. The polynucleotides of the invention can be administered (*e.g.*, RNA, DNA or protein) and introduced into a subject by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention can also be formulated and used as tablets, capsules or

elixirs for oral administration, suppositories for rectal administration, sterile solutions, suspensions for injectable administration, and the other compositions known in the art.

The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, *e.g.*, acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, *e.g.*, systemic administration, into a cell or subject, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (*i.e.*, a cell to which the negatively charged nucleic acid is desirable for delivery). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

By "systemic administration" is meant *in vivo* systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes that lead to systemic absorption include, without limitation: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes exposes the siNA molecules of the invention to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation that can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells, such as cells producing excess BCR-ABL and/or ERG.

By "pharmaceutically acceptable formulation" is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85), which can enhance entry of drugs into the CNS (Joliet-Riant and Tillement, 1999, *Fundam. Clin. Pharmacol.*, 13, 16-26); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery after intracerebral implantation (Emerich, DF *et al.*, 1999, *Cell Transplant*, 8, 47-58) (Alkermes, Inc. Cambridge, MA); and loaded nanoparticles, such as those made of polybutylcyanoacrylate, which can deliver drugs across the blood brain barrier and can alter neuronal uptake mechanisms (*Prog Neuropsychopharmacol Biol Psychiatry*, 23, 941-949, 1999). Other non-limiting examples of delivery strategies for the nucleic acid molecules of the instant invention include material described in Boado *et al.*, 1998, *J. Pharm. Sci.*, 87, 1308-1315; Tyler *et al.*, 1999, *FEBS Lett.*, 421, 280-284; Pardridge *et al.*, 1995, *PNAS USA*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.*, 15, 73-107; Aldrian-Herrada *et al.*, 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler *et al.*, 1999, *PNAS USA*, 96, 7053-7058.

The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic *et al.* *Chem. Rev.* 1995, 95, 2601-2627; Ishiwata *et al.*, *Chem. Pharm. Bull.* 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al.*, *Science* 1995, 267, 1275-1276; Oku *et al.*, 1995, *Biochim. Biophys. Acta*, 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al.*, *J. Biol. Chem.* 1995, 270, 24864-24870; Choi *et al.*, International PCT Publication No. WO 96/10391; Ansell *et al.*, International PCT

Publication No. WO 96/10390; Holland *et al.*, International PCT Publication No. WO 96/10392). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

5       The present invention also includes compositions prepared for storage or administration that include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in  
10       *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

      A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a  
15       disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent  
20       upon potency of the negatively charged polymer.

      The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. The term parenteral as used herein includes percutaneous,  
25       subcutaneous, intravascular (*e.g.*, intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or

adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

5       Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable  
10       excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents; such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or  
15       they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the  
20       active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in a mixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for  
25       example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example



heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions  
5 can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as  
10 liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid

Dispersible powders and granules suitable for preparation of an aqueous suspension by  
15 the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water  
20 emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for  
25 example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a

demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile  
5 injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be  
10 employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The nucleic acid molecules of the invention can also be administered in the form of suppositories, *e.g.*, for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary  
15 temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics,  
20 preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per subject per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated  
25 and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

It is understood that the specific dose level for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body

weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking  
5 water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention can also be administered to a  
10 subject in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

In one embodiment, the invention comprises compositions suitable for administering nucleic acid molecules of the invention to specific cell types. For example, the asialoglycoprotein receptor (ASGPr) (Wu and Wu, 1987, *J. Biol. Chem.* 262, 4429-4432) is  
15 unique to hepatocytes and binds branched galactose-terminal glycoproteins, such as asialoorosomucoid (ASOR). In another example, the folate receptor is overexpressed in many cancer cells. Binding of such glycoproteins, synthetic glycoconjugates, or folates to the receptor takes place with an affinity that strongly depends on the degree of branching of the oligosaccharide chain, for example, triantennary structures are bound with greater affinity  
20 than biantennary or monoantennary chains (Baenziger and Fiete, 1980, *Cell*, 22, 611-620; Connolly *et al.*, 1982, *J. Biol. Chem.*, 257, 939-945). Lee and Lee, 1987, *Glycoconjugate J.*, 4, 317-328, obtained this high specificity through the use of N-acetyl-D-galactosamine as the carbohydrate moiety, which has higher affinity for the receptor, compared to galactose. This "clustering effect" has also been described for the binding and uptake of mannosyl-  
25 terminating glycoproteins or glycoconjugates (Ponpipom *et al.*, 1981, *J. Med. Chem.*, 24, 1388-1395). The use of galactose, galactosamine, or folate based conjugates to transport exogenous compounds across cell membranes can provide a targeted delivery approach to, for example, the treatment of liver disease, cancers of the liver, or other cancers. The use of bioconjugates can also provide a reduction in the required dose of therapeutic compounds

required for treatment. Furthermore, therapeutic bioavailability, pharmacodynamics, and pharmacokinetic parameters can be modulated through the use of nucleic acid bioconjugates of the invention. Non-limiting examples of such bioconjugates are described in Vargeese *et al.*, USSN 10/201,394, filed August 13, 2001; and Matulic-Adamic *et al.*, USSN 60/362,016,  
5 filed March 6, 2002.

Alternatively, certain siNA molecules of the instant invention can be expressed within cells from eukaryotic promoters (e.g., Izant and Weintraub, 1985, *Science*, 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci. USA* 83, 399; Scanlon *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88, 10591-5; Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2,  
10 3-15; Dropulic *et al.*, 1992, *J. Virol.*, 66, 1432-41; Weerasinghe *et al.*, 1991, *J. Virol.*, 65, 5531-4; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Sarver *et al.*, 1990 *Science*, 247, 1222-1225; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Good *et al.*, 1997, *Gene Therapy*, 4, 45. Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the  
15 appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a enzymatic nucleic acid (Draper *et al.*, PCT WO 93/23569, and Sullivan *et al.*, PCT WO 94/02595; Ohkawa *et al.*, 1992, *Nucleic Acids Symp. Ser.*, 27, 15-6; Taira *et al.*, 1991, *Nucleic Acids Res.*, 19, 5125-30; Ventura *et al.*, 1993, *Nucleic Acids Res.*, 21, 3249-55; Chowrira *et al.*, 1994, *J. Biol. Chem.*, 269, 25856.

20 In another aspect of the invention, RNA molecules of the present invention can be expressed from transcription units (see for example Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. In another embodiment, pol III based  
25 constructs are used to express nucleic acid molecules of the invention (see for example Thompson, U.S. Pats. Nos. 5,902,880 and 6,146,886). The recombinant vectors capable of expressing the siNA molecules can be delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly administered as necessary. Once expressed,

the siNA molecule interacts with the target mRNA and generates an RNAi response. Delivery of siNA molecule expressing vectors can be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for  
5 introduction into the desired target cell (for a review see Couture *et al.*, 1996, *TIG.*, 12, 510).

In one aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the instant invention. The expression vector can encode one or both strands of a siNA duplex, or a single self-complementary strand that self hybridizes into a siNA duplex. The nucleic acid sequences encoding the  
10 siNA molecules of the instant invention can be operably linked in a manner that allows expression of the siNA molecule (see for example Paul *et al.*, 2002, *Nature Biotechnology*, 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee *et al.*, 2002, *Nature Biotechnology*, 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725).

15 In another aspect, the invention features an expression vector comprising: a) a transcription initiation region (e.g., eukaryotic pol I, II or III initiation region); b) a transcription termination region (e.g., eukaryotic pol I, II or III termination region); and c) a nucleic acid sequence encoding at least one of the siNA molecules of the instant invention; wherein said sequence is operably linked to said initiation region and said termination region,  
20 in a manner that allows expression and/or delivery of the siNA molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the siNA of the invention; and/or an intron (intervening sequences).

Transcription of the siNA molecule sequences can be driven from a promoter for  
25 eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type depends on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme

is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res.*, 21, 2867-72; Lieber *et al.*, 1993, *Methods Enzymol.*, 217, 47-66; Zhou *et al.*, 1990, *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that nucleic acid molecules expressed from such promoters  
5 can function in mammalian cells (e.g. Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. U S A*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Yu *et al.*, 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier *et al.*, 1992, *EMBO J.*, 11, 4411-8; Lisiewicz *et al.*, 1993, *Proc. Natl. Acad. Sci. U. S. A*, 90, 8000-4; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Sullenger & Cech,  
10 1993, *Science*, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as siNA in cells (Thompson *et al.*, *supra*; Couture and Stinchcomb, 1996, *supra*; Noonberg *et al.*, 1994, *Nucleic Acid Res.*, 22, 2830; Noonberg *et al.*, U.S. Pat. No. 5,624,803; Good *et al.*, 1997,  
15 *Gene Ther.*, 4, 45; Beigelman *et al.*, International PCT Publication No. WO 96/18736. The above siNA transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 1996, *supra*).

20 In another aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the siNA molecules of the invention in a manner that allows expression of that siNA molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; and c) a nucleic acid sequence encoding at least one strand of the siNA molecule, wherein the  
25 sequence is operably linked to the initiation region and the termination region in a manner that allows expression and/or delivery of the siNA molecule.

In another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; and d) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably

linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the open reading frame and the termination region in a manner that allows expression and/or delivery of the siNA molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; and d) a nucleic acid sequence encoding at least one siNA molecule, wherein the sequence is operably linked to the initiation region, the intron and the termination region in a manner which allows expression and/or delivery of the nucleic acid molecule.

In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; and e) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the intron, the open reading frame and the termination region in a manner which allows expression and/or delivery of the siNA molecule.

#### BCR-ABL biology and biochemistry

Transformation is a cumulative process whereby normal control of cell growth and differentiation is interrupted, usually through the accumulation of mutations affecting the expression of genes that regulate cell growth and differentiation. More than 70% of hematopoietic malignancies have been shown to possess recurrent chromosomal translocations. The underlying mechanism of chromosomal translocation can be classified as either gene fusion or transcriptional deregulation. The gene fusion mechanism involves two genes that are joined into one, resulting in a chimeric RNA transcript which makes a chimeric protein product. Since the chimeric protein is not found in any normal tissue, it can serve as a tumor specific marker in identifying disease. A related change in protein function can confer a growth advantage leading to malignant transformation. Non-limiting examples of gene fusion products include BCR-ABL, PML-RAR-alpha, and MLL/LTG4, 9, 19. The transcriptional deregulation mechanism does not involve the generation of chimeric protein, but rather juxtaposes one gene to a target gene, thereby transcriptionally deregulating the

target gene. This type of translocation is frequently found in lymphomas, such as the *Myc* translocation in Burkitt's lymphoma; the *BCL2* translocation in follicular lymphoma; and *BCL1* in mantle cell lymphoma.

Chronic myelogenous leukemia (also called chronic myeloid leukemia or CML) exhibits a characteristic disease course, presenting initially as a chronic granulocytic hyperplasia, and invariably evolving into an acute leukemia which is caused by the clonal expansion of a cell with a less differentiated phenotype, resulting in the blast crisis stage of the disease. CML is an unstable disease that ultimately progresses to a terminal stage which resembles acute leukemia. This lethal disease affects approximately 16,000 patients a year. Chemotherapeutic agents, such as hydroxyurea or busulfan, can reduce the leukemic burden but do not impact the life expectancy of the patient (which is approximately 4 years). Consequently, CML patients are candidates for bone marrow transplantation (BMT) therapy. However, for those patients who survive BMT, disease recurrence remains a major obstacle.

The Philadelphia (Ph) chromosome which results from the translocation of the *abl* oncogene from chromosome 9 to the *BCR* gene on chromosome 22 is found in greater than 95% of CML patients and in 10-25% of all cases of acute lymphoblastic leukemia. In virtually all Ph-positive CMLs and approximately 50% of the Ph-positive ALLs, the leukemic cells express BCR-ABL fusion mRNAs in which exon 2 (b2-a2 junction) or exon 3 (b3-a2 junction) from the major breakpoint cluster region of the *BCR* gene is spliced to exon 2 of the *ABL* gene. In the remaining cases of Ph-positive ALL, the first exon of the *BCR* gene is spliced to exon 2 of the *ABL* gene. The b3-a2 and b2-a2 fusion mRNAs encode 210 kd BCR-ABL fusion proteins which exhibit oncogenic activity through increased tyrosine kinase activity. The BCR-ABL tyrosine kinase elicits oncogenic transformation through the constitutive stimulation of specific signal transduction pathways. Several mechanisms have been proposed to explain how BCR-ABL transforms cells. For example, BCR-ABL has been shown to block apoptosis, increase cell proliferation, alter cell adhesion and increase cell motility.

With the exception of CML, chronic myeloproliferative disorders (CMPDs) are a heterogeneous spectrum of conditions for which the molecular pathogenesis is not well



- understood. Most cases have a normal or aneuploid karyotype, but a minority present with a reciprocal translocation that disrupts specific tyrosine kinase genes, most commonly PDGFRB or FGFR1. These translocations result in the production of constitutively active tyrosine kinase fusion proteins that deregulate hemopoiesis in a manner analogous to BCR-ABL. The chimeric product type of translocation in acute promyelocytic leukemia, which has t(15;17)(q22; q21), involves the promyelocytic leukemia (PML) gene. Although the function of PML still remains to be elucidated, the translocation to the Retinoid receptor A interrupts its regulatory region, resulting in deregulation of gene function, most likely through the differentiation block at a stage where this function is required.
- 10 The use of small interfering nucleic acid molecules targeting chromosomal translocation genes therefore provides a useful class of novel therapeutic agents that can be used in the treatment of leukemias, lymphomas and/or any other disease or condition that can result from chromosomal translocation events.

#### ERG biology and biochemistry

- 15 ERG is a member of the Ets oncogene superfamily of transcription factors which share common DNA binding domains yet differ in their transactivation domains. The Ets family of transcription factors are implicated in the control of the constitutive expression of a wide variety of genes. In hematopoietic cells, the Ets family appears to be important in the early stages of lymphocyte cell-type specification. ERG has been identified during arrayed cDNA library screens for genes encoding transcription factors expressed specifically during T cell lineage commitment. ERG expression is induced during T-cell lineage specification and is subsequently silenced permanently (Anderson *et al.*, 1999, *Development*, 126(14), 3131-3148). ERG is rearranged in human myeloid leukemia with t(16;21) chromosomal translocation. This rearrangement generates the TLS-ERG oncogene which is associated with poor prognosis human acute myeloid leukemia (AML), secondary AML associated with myelodysplastic syndrome (MDS), and chronic myeloid leukemia (CML) in blast crisis (Kong *et al.*, 1997, *Blood*, 90, 1192-1199). The altered transcriptional activating and DNA-binding activities of the TLS-ERG gene product are implicated in the genesis or progression of t(16;21)-associated human myeloid leukemias (Prasad *et al.*, 1994, *Oncogene*, 9, 3717-

3729). In addition, retroviral transduction of TLS-ERG has been shown to initiate a leukemogenic program in normal human hematopoietic cells (Pereira *et al.*, 1998, *PNAS USA*, 95, 8239-8244).

The expression of several members of the Ets family of transcription factors, including  
5 ERG, correlates with the occurrence of invasive processes such as angiogenesis, including  
endothelial cell proliferation, endothelial cell differentiation, and matrix metalloproteinase  
transduction, during normal and pathological development (for review see Mattot *et al.*,  
1999, *J. Soc. Biol.*, 193(2), 147-153 and Soncin *et al.*, 1999, *Pathol. Biol.*, 47(4), 358-363).  
Ets family transcription factors, including ERG, have been implicated in the upregulation of  
10 human heme oxygenase gene expression. Overexpression of human heme oxygenase-1 has  
been shown to have the potential to promote endothelial cell proliferation and angiogenesis.  
Ets binding sites in regulatory sequences of heme oxygenase-1 have been identified. As  
such, Ets family transcriptional regulation of human heme oxygenase may play an important  
role in coronary collateral circulation, tumor growth, angiogenesis, and hemoglobin induced  
15 endothelial cell injury (Deramandt *et al.*, 1999, *J. Cell. Biochem.*, 72(3), 311-321).

The Ets, Fos, and Jun transcription factors control the expression of stromelysin-1 and  
collagenase-1 genes that encode two matrix metalloproteinases implicated in normal growth  
and development, as well as in tumor invasion and metastasis. It has been shown that the Ets  
transcription factors interact with each other and with the c-Fos/c-Jun complex via distinct  
20 protein domains in both a DNA-dependent and independent manner (Basuyaux *et al.*, 1997,  
*J. Biol. Chem.*, 272(42), 26188-95). Moreover, ERG activates collagenase-1 gene by  
physically interacting with c-Fos/c-Jun (Buttice *et al.*, 1996, *Oncogene*, 13(11), 2297-2306).  
Altered expression of ERG is associated with genetic translocations on chromosome 21 in  
immortal and cervical carcinoma cell lines (Simpson *et al.*, 1997, *Oncogene*, 14(18), 2149-  
25 2157). An additional translocation fusion product of ERG, EWS-ERG, has been identified in  
a large proportion of Ewing family tumors as a transcriptional activator (Sorensen *et al.*,  
1994, *Nat. Genet.*, 6(2), 146-151). Expression of the EWS-ERG fusion protein has been  
shown to be essential for maintaining the oncogenic and tumorigenic properties of certain  
human tumor cells via inhibition of apoptosis (Yi *et al.*, 1997, *Oncogene*, 14(11), 1259-

1268). Hart *et al.*, 1995, *Oncogene*, 10(7), 1423-30, describe human ERG as a proto-oncogene with mitogenic and transforming activity. Transfection of NIH3T3 cells with an ERG expression construct driven by the sheep metallothionein 1a promoter (sMTERG) results in cells that become morphologically altered, non-serum and non-anchorage  
5 dependant, and result in the formation of solid tumors when injected in nude mice (Hart *et al.*, *supra*).

The endothelium, which lines the blood vessels and acts as a barrier between blood and tissues, plays an important role in maintaining vascular homeostasis. The endothelium regulates processes such as leukocyte infiltration, coagulation, and maintains the integrity of  
10 cell-cell junctions. Proliferation of endothelial cells, which occurs in angiogenesis, is a tightly controlled process that can occur in a physiological state (e.g. in wound healing and the menstrual cycle) but also occurs in a disease. Endothelial activation is involved in diseases such as cancer and metastasis, rheumatoid arthritis, cataract formation, atherosclerosis, thrombosis and many others. Inflammatory mediators such as the pleiotropic  
15 cytokine TNF-alpha alter the resting phenotype of the endothelium such that it becomes pro-inflammatory, pro-thrombotic and often pro-angiogenic. The ensuing changes in gene regulation have been extensively studied and involve the up-regulation of inflammatory cell adhesion molecules ICAM-1, E-selectin and VCAM-1 and pro-thrombotic proteins such as tissue factor, both in vitro and in vivo (McEver, 1991, *Thrombosis and Haemostasis*, 65, 223;  
20 Saadi *et al.*, 1995, *J. Exp. Med.*, 182, 1807). The role of TNF-alpha in modulating angiogenesis has been demonstrated in vivo but the evidence of an effect in vitro is less clear and in some cases conflicting. TNF-alpha is pro-angiogenic in rabbit corneal and chick chorioallantoic membrane in vivo models (Frater-Schroder *et al.*, 1987, *PNAS USA*, 84, 5277; Leibovich *et al.*, 1987, *Nature*, 329, 630) and more recently in rheumatoid arthritis  
25 patients, anti-TNF-alpha therapy decreased circulating levels of vascular endothelial growth factor (VEGF) (Paleolog, 1997, *Molecular Pathology*, 50, 225). In vitro, TNF-alpha can induce basic fibroblast growth factor (bFGF), platelet activated factor (PAF) and urokinase-type plasminogen activator (u-TPA), all of which are angiogenic and increase transcription of the VEGF receptor (VEGFR-2). On the contrary, TNF-alpha can also inhibit endothelial cell  
30 proliferation in vitro and cause tumor regression (Carswell *et al.*, 1975, *PNAS USA*, 72,

3666). The mechanisms by which TNF-alpha mediates these effects on cell proliferation/angiogenesis are unclear and may involve regulation of genes which are not involved in the pro-inflammatory mode of action of this cytokine.

Studies on the effects of TNF-alpha on endothelial genes have shown that TNF-alpha  
5 down-regulates the transcription factor ERG in human umbilical vein endothelial cells (HUVEC) (McLaughlin *et al.*, 1999, *J. of Cell Science*, 112, 4695). ERG is a member of the Ets family of transcription factors which play roles in embryonic development, inflammation, and cellular transformation. An 85 amino acid Ets domain is conserved throughout the family and is necessary for binding a GGAA core DNA binding site. ERG is a  
10 proto-oncogene as shown by the ability of NIH3T3 cells overexpressing ERG to form solid tumors in nude mice. Although downstream targets of ERG have not been clearly identified, in vitro evidence exists which suggests that an ERG cDNA can transactivate the vWF, ICAM-2, VE-Cadherin and collagenase promoters using reporter gene assays and purified ERG/GST protein or ERG from endothelial cell nuclear extracts can bind to the VE-  
15 Cadherin, stromelysin and vWF promoter Ets sites (McLaughlin *et al.*, *supra*).

#### Examples:

The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

#### Example 1: Tandem synthesis of siNA constructs

20 Exemplary siNA molecules of the invention are synthesized in tandem using a cleavable linker, for example, a succinyl-based linker. Tandem synthesis as described herein is followed by a one-step purification process that provides RNAi molecules in high yield. This approach is highly amenable to siNA synthesis in support of high throughput RNAi screening, and can be readily adapted to multi-column or multi-well synthesis platforms.

25 After completing a tandem synthesis of a siNA oligo and its complement in which the 5'-terminal dimethoxytrityl (5'-O-DMT) group remains intact (trityl on synthesis), the oligonucleotides are deprotected as described above. Following deprotection, the siNA

sequence strands are allowed to spontaneously hybridize. This hybridization yields a duplex in which one strand has retained the 5'-O-DMT group while the complementary strand comprises a terminal 5'-hydroxyl. The newly formed duplex behaves as a single molecule during routine solid-phase extraction purification (Trityl-On purification) even though only  
5 one molecule has a dimethoxytrityl group. Because the strands form a stable duplex, this dimethoxytrityl group (or an equivalent group, such as other trityl groups or other hydrophobic moieties) is all that is required to purify the pair of oligos, for example, by using a C18 cartridge.

Standard phosphoramidite synthesis chemistry is used up to the point of introducing a  
10 tandem linker, such as an inverted deoxy abasic succinate or glyceryl succinate linker (see Figure 1) or an equivalent cleavable linker. A non-limiting example of linker coupling conditions that can be used includes a hindered base such as diisopropylethylamine (DIPA) and/or DMAP in the presence of an activator reagent such as Bromotripyrrolidinophosphoniumhexafluorophosphate (PyBrOP). After the linker is  
15 coupled, standard synthesis chemistry is utilized to complete synthesis of the second sequence leaving the terminal the 5'-O-DMT intact. Following synthesis, the resulting oligonucleotide is deprotected according to the procedures described herein and quenched with a suitable buffer, for example with 50mM NaOAc or 1.5M  $\text{NH}_4\text{H}_2\text{CO}_3$ .

Purification of the siNA duplex can be readily accomplished using solid phase  
20 extraction, for example using a Waters C18 SepPak 1g cartridge conditioned with 1 column volume (CV) of acetonitrile, 2 CV  $\text{H}_2\text{O}$ , and 2 CV 50mM NaOAc. The sample is loaded and then washed with 1 CV  $\text{H}_2\text{O}$  or 50mM NaOAc. Failure sequences are eluted with 1 CV 14% ACN (Aqueous with 50mM NaOAc and 50mM NaCl). The column is then washed, for example with 1 CV  $\text{H}_2\text{O}$  followed by on-column detritylation, for example by passing 1 CV  
25 of 1% aqueous trifluoroacetic acid (TFA) over the column, then adding a second CV of 1% aqueous TFA to the column and allowing to stand for approximately 10 minutes. The remaining TFA solution is removed and the column washed with  $\text{H}_2\text{O}$  followed by 1 CV 1M NaCl and additional  $\text{H}_2\text{O}$ . The siNA duplex product is then eluted, for example, using 1 CV 20% aqueous CAN.

Figure 2 provides an example of MALDI-TOV mass spectrometry analysis of a purified siNA construct in which each peak corresponds to the calculated mass of an individual siNA strand of the siNA duplex. The same purified siNA provides three peaks when analyzed by capillary gel electrophoresis (CGE), one peak presumably corresponding to the duplex siNA, and two peaks presumably corresponding to the separate siNA sequence strands. Ion exchange HPLC analysis of the same siNA contract only shows a single peak. Testing of the purified siNA construct using a luciferase reporter assay described below demonstrated the same RNAi activity compared to siNA constructs generated from separately synthesized oligonucleotide sequence strands.

10 Example 2: Identification of potential siNA target sites in any RNA sequence

The sequence of an RNA target of interest, such as a viral or human mRNA transcript, is screened for target sites, for example, by using a computer folding algorithm. In a non-limiting example, the sequence of a gene or RNA gene transcript derived from a database, such as Genbank, is used to generate siNA targets having complementarity to the target.

15 Such sequences can be obtained from a database, or can be determined experimentally as known in the art. Target sites that are known, for example, those target sites determined to be effective target sites based on studies with other nucleic acid molecules, for example ribozymes or antisense, or those targets known to be associated with a disease or condition such as those sites containing mutations or deletions, can be used to design siNA molecules

20 targeting those sites. Various parameters can be used to determine which sites are the most suitable target sites within the target RNA sequence. These parameters include but are not limited to secondary or tertiary RNA structure, the nucleotide base composition of the target sequence, the degree of homology between various regions of the target sequence, or the relative position of the target sequence within the RNA transcript. Based on these

25 determinations, any number of target sites within the RNA transcript can be chosen to screen siNA molecules for efficacy, for example by using *in vitro* RNA cleavage assays, cell culture, or animal models. In a non-limiting example, anywhere from 1 to 1000 target sites are chosen within the transcript based on the size of the siNA construct to be used. High throughput screening assays can be developed for screening siNA molecules using methods

known in the art, such as with multi-well or multi-plate assays to determine efficient reduction in target gene expression.

Example 3: Selection of siNA molecule target sites in a RNA

5 The following non-limiting steps can be used to carry out the selection of siNAs targeting a given gene sequence or transcript.

1. The target sequence is parsed *in silico* into a list of all fragments or subsequences of a particular length, for example 23 nucleotide fragments, contained within the target sequence. This step is typically carried out using a custom Perl script, but commercial sequence analysis programs such as Oligo, MacVector, or the GCG Wisconsin Package  
10 can be employed as well.
2. In some instances the siNAs correspond to more than one target sequence; such would be the case for example in targeting different transcripts of the same gene, targeting different transcripts of more than one gene, or for targeting both the human gene and an animal homolog. In this case, a subsequence list of a particular length is generated for each of  
15 the targets, and then the lists are compared to find matching sequences in each list. The subsequences are then ranked according to the number of target sequences that contain the given subsequence; the goal is to find subsequences that are present in most or all of the target sequences. Alternately, the ranking can identify subsequences that are unique to a target sequence, such as a mutant target sequence. Such an approach would enable  
20 the use of siNA to target specifically the mutant sequence and not effect the expression of the normal sequence.
3. In some instances the siNA subsequences are absent in one or more sequences while present in the desired target sequence; such would be the case if the siNA targets a gene with a paralogous family member that is to remain untargeted. As in case 2 above, a  
25 subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find sequences that are present in the target gene but are absent in the untargeted paralog.

4. The ranked siNA subsequences can be further analyzed and ranked according to GC content. A preference can be given to sites containing 30-70% GC, with a further preference to sites containing 40-60% GC.
5. The ranked siNA subsequences can be further analyzed and ranked according to self-folding and internal hairpins. Weaker internal folds are preferred; strong hairpin structures are to be avoided.
6. The ranked siNA subsequences can be further analyzed and ranked according to whether they have runs of GGG or CCC in the sequence. GGG (or even more Gs) in either strand can make oligonucleotide synthesis problematic and can potentially interfere with RNAi activity, so it is avoided whenever better sequences are available. CCC is searched in the target strand because that will place GGG in the antisense strand.
7. The ranked siNA subsequences can be further analyzed and ranked according to whether they have the dinucleotide UU (uridine dinucleotide) on the 3'-end of the sequence, and/or AA on the 5'-end of the sequence (to yield 3' UU on the antisense sequence). These sequences allow one to design siNA molecules with terminal TT thymidine dinucleotides.
8. Four or five target sites are chosen from the ranked list of subsequences as described above. For example, in subsequences having 23 nucleotides, the right 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the upper (sense) strand of the siNA duplex, while the reverse complement of the left 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the lower (antisense) strand of the siNA duplex (see Tables II and III). If terminal TT residues are desired for the sequence (as described in paragraph 7), then the two 3' terminal nucleotides of both the sense and antisense strands are replaced by TT prior to synthesizing the oligos.
9. The siNA molecules are screened in an *in vitro*, cell culture or animal model system to identify the most active siNA molecule or the most preferred target site within the target RNA sequence.



In an alternate approach, a pool of siNA constructs specific to a BCR-ABL and/or ERG target sequence is used to screen for target sites in cells expressing BCR-ABL and/or ERG RNA, such as human cultured chronic myelogenous leukemic cells (e.g., K562, HUVEC or HeLa cells). The general strategy used in this approach is shown in Figure 9.

- 5 A non-limiting example of such is a pool comprising sequences having sense sequences comprising SEQ ID NOs. 1-263, 527-845, 1165-1182, 1201-1218, 1589-1596, 1601-1604, 1609-1612, 1617-1620, 1625-1628, 1632-1636, 1641-1644, 1237-1412, 1597-1600, 1649-1652, 1657-1660, and 1665-1668 and antisense sequences comprising SEQ ID NOs. 264-526, 846-1164, 1183-1200, 1219-1236, 1605-1608, 1613-1616, 1621-1624, 1629-1631, 10 1637-1640, 1645-1648, 1413-1588, 1653-1656, 1661-1664, and 1669-1672 respectively. K562, HUVEC or HeLa cells expressing BCR-ABL and/or ERG are transfected with the pool of siNA constructs and cells that demonstrate a phenotype associated with BCR-ABL and/or ERG inhibition are sorted. The pool of siNA constructs can be expressed from transcription cassettes inserted into appropriate vectors (see for example Figure 7 and Figure 15 8). The siNA from cells demonstrating a positive phenotypic change (e.g., decreased proliferation, decreased BCR-ABL and/or ERG mRNA levels or decreased BCR-ABL and/or ERG protein expression), are sequenced to determine the most suitable target site(s) within the target BCR-ABL and/or ERG RNA sequence.

Example 4: BCR-ABL and/or ERG targeted siNA design

- 20 siNA target sites were chosen by analyzing sequences of the BCR-ABL and/or ERG RNA target and optionally prioritizing the target sites on the basis of folding (structure of any given sequence analyzed to determine siNA accessibility to the target), by using a library of siNA molecules as described in Example 3, or alternately by using an *in vitro* siNA system as described in Example 6 herein. siNA molecules were designed that could bind each target and are optionally individually analyzed by computer folding to assess whether the siNA 25 molecule can interact with the target sequence. Varying the length of the siNA molecules can be chosen to optimize activity. Generally, a sufficient number of complementary nucleotide bases are chosen to bind to, or otherwise interact with, the target RNA, but the degree of complementarity can be modulated to accommodate siNA duplexes or varying

length or base composition. By using such methodologies, siNA molecules can be designed to target sites within any known RNA sequence, for example those RNA sequences corresponding to the any gene transcript.

Chemically modified siNA constructs are designed to provide nuclease stability for systemic administration in vivo and/or improved pharmacokinetic, localization, and delivery properties while preserving the ability to mediate RNAi activity. Chemical modifications as described herein are introduced synthetically using synthetic methods described herein and those generally known in the art. The synthetic siNA constructs are then assayed for nuclease stability in serum and/or cellular/tissue extracts (e.g. liver extracts). The synthetic siNA constructs are also tested in parallel for RNAi activity using an appropriate assay, such as a luciferase reporter assay as described herein or another suitable assay that can quantify RNAi activity. Synthetic siNA constructs that possess both nuclease stability and RNAi activity can be further modified and re-evaluated in stability and activity assays. The chemical modifications of the stabilized active siNA constructs can then be applied to any siNA sequence targeting any chosen RNA and used, for example, in target screening assays to pick lead siNA compounds for therapeutic development (see for example Figure 11).

#### Example 5: Chemical Synthesis and Purification of siNA

siNA molecules can be designed to interact with various sites in the RNA message, for example, target sequences within the RNA sequences described herein. The sequence of one strand of the siNA molecule(s) is complementary to the target site sequences described above. The siNA molecules can be chemically synthesized using methods described herein. Inactive siNA molecules that are used as control sequences can be synthesized by scrambling the sequence of the siNA molecules such that it is not complementary to the target sequence. Generally, siNA constructs can be synthesized using solid phase oligonucleotide synthesis methods as described herein (see for example Usman *et al.*, US Patent Nos. 5,804,683; 5,831,071; 5,998,203; 6,117,657; 6,353,098; 6,362,323; 6,437,117; 6,469,158; Scaringe *et al.*, US Patent Nos. 6,111,086; 6,008,400; 6,111,086 all incorporated by reference herein in their entirety).

In a non-limiting example, RNA oligonucleotides are synthesized in a stepwise fashion using the phosphoramidite chemistry as is known in the art. Standard phosphoramidite chemistry involves the use of nucleosides comprising any of 5'-O-dimethoxytrityl, 2'-O-tert-butyl dimethylsilyl, 3'-O-2-Cyanoethyl N,N-diisopropylphosphoramide groups, and exocyclic amine protecting groups (e.g. N6-benzoyl adenosine, N4 acetyl cytidine, and N2-isobutyryl guanosine). Alternately, 2'-O-Silyl Ethers can be used in conjunction with acid-labile 2'-O-orthoester protecting groups in the synthesis of RNA as described by Scaringe *supra*. Differing 2' chemistries can require different protecting groups, for example 2'-deoxy-2'-amino nucleosides can utilize N-phthaloyl protection as described by Usman *et al.*,  
5  
10 US Patent 5,631,360, incorporated by reference herein in its entirety).

During solid phase synthesis, each nucleotide is added sequentially (3' - to 5'-direction) to the solid support-bound oligonucleotide. The first nucleoside at the 3'-end of the chain is covalently attached to a solid support (e.g., controlled pore glass or polystyrene) using various linkers. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator  
15 are combined resulting in the coupling of the second nucleoside phosphoramidite onto the 5'-end of the first nucleoside. The support is then washed and any unreacted 5'-hydroxyl groups are capped with a capping reagent such as acetic anhydride to yield inactive 5'-acetyl moieties. The trivalent phosphorus linkage is then oxidized to a more stable phosphate linkage. At the end of the nucleotide addition cycle, the 5'-O-protecting group is cleaved  
20 under suitable conditions (e.g., acidic conditions for trityl-based groups and Fluoride for silyl-based groups). The cycle is repeated for each subsequent nucleotide.

Modification of synthesis conditions can be used to optimize coupling efficiency, for example by using differing coupling times, differing reagent/phosphoramidite concentrations, differing contact times, differing solid supports and solid support linker chemistries  
25 depending on the particular chemical composition of the siNA to be synthesized. Deprotection and purification of the siNA can be performed as is generally described in Usman *et al.*, US 5,831,071, US 6,353,098, US 6,437,117, and Bellon *et al.*, US 6,054,576, US 6,162,909, US 6,303,773, incorporated by reference herein in their entirety or Scaringe *supra*. Additionally, deprotection conditions can be modified to provide the best possible

yield and purity of siNA constructs. For example, applicant has observed that oligonucleotides comprising 2'-deoxy-2'-fluoro nucleotides can degrade under inappropriate deprotection conditions. Such oligonucleotides are deprotected using aqueous methylamine at about 35°C for 30 minutes. If the 2'-deoxy-2'-fluoro containing oligonucleotide also  
5 comprises ribonucleotides, after deprotection with aqueous methylamine at about 35°C for 30 minutes, TEA-HF is added and the reaction maintained at about 65°C for an additional 15 minutes.

Example 6: RNAi *in vitro* assay to assess siNA activity

An *in vitro* assay that recapitulates RNAi in a cell-free system is used to evaluate siNA  
10 constructs targeting BCR-ABL and/or ERG RNA targets. The assay comprises the system described by Tuschl *et al.*, 1999, *Genes and Development*, 13, 3191-3197 and Zamore *et al.*, 2000, *Cell*, 101, 25-33 adapted for use with BCR-ABL and/or ERG target RNA. A *Drosophila* extract derived from syncytial blastoderm is used to reconstitute RNAi activity *in vitro*. Target RNA is generated via *in vitro* transcription from an appropriate BCR-ABL  
15 and/or ERG expressing plasmid using T7 RNA polymerase or via chemical synthesis as described herein. Sense and antisense siNA strands (for example 20 uM each) are annealed by incubation in buffer (such as 100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 min. at 90°C followed by 1 hour at 37°C, then diluted in lysis buffer (for example 100 mM potassium acetate, 30 mM HEPES-KOH at pH 7.4, 2mM  
20 magnesium acetate). Annealing can be monitored by gel electrophoresis on an agarose gel in TBE buffer and stained with ethidium bromide. The *Drosophila* lysate is prepared using zero to two-hour-old embryos from Oregon R flies collected on yeasted molasses agar that are dechorionated and lysed. The lysate is centrifuged and the supernatant isolated. The assay comprises a reaction mixture containing 50% lysate [vol/vol], RNA (10-50 pM final  
25 concentration), and 10% [vol/vol] lysis buffer containing siNA (10 nM final concentration). The reaction mixture also contains 10 mM creatine phosphate, 10 ug/ml creatine phosphokinase, 100 uM GTP, 100 uM UTP, 100 uM CTP, 500 uM ATP, 5 mM DTT, 0.1 U/uL RNasin (Promega), and 100 uM of each amino acid. The final concentration of potassium acetate is adjusted to 100 mM. The reactions are pre-assembled on ice and

preincubated at 25° C for 10 minutes before adding RNA, then incubated at 25° C for an additional 60 minutes. Reactions are quenched with 4 volumes of 1.25 x Passive Lysis Buffer (Promega). Target RNA cleavage is assayed by RT-PCR analysis or other methods known in the art and are compared to control reactions in which siNA is omitted from the  
5 reaction.

Alternately, internally-labeled target RNA for the assay is prepared by *in vitro* transcription in the presence of [ $\alpha$ - $^{32}$ P] CTP, passed over a G 50 Sephadex column by spin chromatography and used as target RNA without further purification. Optionally, target RNA is 5'- $^{32}$ P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed as  
10 described above and target RNA and the specific RNA cleavage products generated by RNAi are visualized on an autoradiograph of a gel. The percentage of cleavage is determined by Phosphor Imager<sup>®</sup> quantitation of bands representing intact control RNA or RNA from control reactions without siNA and the cleavage products generated by the assay.

In one embodiment, this assay is used to determine target sites the BCR-ABL and/or  
15 ERG RNA target for siNA mediated RNAi cleavage, wherein a plurality of siNA constructs are screened for RNAi mediated cleavage of the BCR-ABL and/or ERG RNA target, for example, by analyzing the assay reaction by electrophoresis of labeled target RNA, or by northern blotting, as well as by other methodology well known in the art.

Example 7: Nucleic acid inhibition of BCR-ABL and/or ERG target RNA *in vivo*

20 siNA molecules targeted to the human BCR-ABL and/or ERG RNA are designed and synthesized as described above. These nucleic acid molecules can be tested for cleavage activity *in vivo*, for example, using the following procedure. The target sequences and the nucleotide location within the BCR-ABL and/or ERG RNA are given in Table II and III.

Two formats are used to test the efficacy of siNAs targeting BCR-ABL and/or ERG.  
25 First, the reagents are tested in cell culture using, for example, cultured chronic myelogenous leukemic cells (e.g., K562, HUVEC or HeLa cells) to determine the extent of RNA and protein inhibition. siNA reagents (e.g.; see Tables II and III) are selected against the BCR-

ABL and/or ERG target as described herein. RNA inhibition is measured after delivery of these reagents by a suitable transfection agent to, for example, K562, HUVEC or HeLa cells. Relative amounts of target RNA are measured versus actin using real-time PCR monitoring of amplification (eg., ABI 7700 Taqman®). A comparison is made to a mixture of  
5 oligonucleotide sequences made to unrelated targets or to a randomized siNA control with the same overall length and chemistry, but randomly substituted at each position. Primary and secondary lead reagents are chosen for the target and optimization performed. After an optimal transfection agent concentration is chosen, a RNA time-course of inhibition is performed with the lead siNA molecule. In addition, a cell-plating format can be used to  
10 determine RNA inhibition.

#### Delivery of siNA to Cells

Cells (e.g., K562, HUVEC or HeLa cells) are seeded, for example, at  $1 \times 10^5$  cells per well of a six-well dish in EGM-2 (BioWhittaker) the day before transfection. siNA (final concentration, for example 20nM) and cationic lipid (e.g., final concentration  $2 \mu\text{g/ml}$ ) are  
15 complexed in EGM basal media (Biowhittaker) at  $37^\circ\text{C}$  for 30 mins in polystyrene tubes. Following vortexing, the complexed siNA is added to each well and incubated for the times indicated. For initial optimization experiments, cells are seeded, for example, at  $1 \times 10^3$  in 96 well plates and siNA complex added as described. Efficiency of delivery of siNA to cells is determined using a fluorescent siNA complexed with lipid. Cells in 6-well dishes are  
20 incubated with siNA for 24 hours, rinsed with PBS and fixed in 2% paraformaldehyde for 15 minutes at room temperature. Uptake of siNA is visualized using a fluorescent microscope.

#### Taqman and Lightcycler quantification of mRNA

Total RNA is prepared from cells following siNA delivery, for example, using Qiagen RNA purification kits for 6-well or Rneasy extraction kits for 96-well assays. For Taqman  
25 analysis, dual-labeled probes are synthesized with the reporter dye, FAM or JOE, covalently linked at the 5'-end and the quencher dye TAMRA conjugated to the 3'-end. One-step RT-PCR amplifications are performed on, for example, an ABI PRISM 7700 Sequence Detector using 50  $\mu\text{l}$  reactions consisting of 10  $\mu\text{l}$  total RNA, 100 nM forward primer, 900 nM reverse

primer, 100 nM probe, 1X TaqMan PCR reaction buffer (PE-Applied Biosystems), 5.5 mM MgCl<sub>2</sub>, 300 μM each dATP, dCTP, dGTP, and dTTP, 10U RNase Inhibitor (Promega), 1.25U AmpliTaq Gold (PE-Applied Biosystems) and 10U M-MLV Reverse Transcriptase (Promega). The thermal cycling conditions can consist of 30 min at 48°C, 10 min at 95°C, 5 followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. Quantitation of mRNA levels is determined relative to standards generated from serially diluted total cellular RNA (300, 100, 33, 11 ng/rxn) and normalizing to β-actin or GAPDH mRNA in parallel TaqMan reactions. For each gene of interest an upper and lower primer and a fluorescently labeled probe are designed. Real time incorporation of SYBR Green I dye into a specific PCR product can be 10 measured in glass capillary tubes using a lightcycler. A standard curve is generated for each primer pair using control cRNA. Values are represented as relative expression to GAPDH in each sample.

#### Western blotting

Nuclear extracts can be prepared using a standard micro preparation technique (see for 15 example Andrews and Faller, 1991, *Nucleic Acids Research*, 19, 2499). Protein extracts from supernatants are prepared, for example using TCA precipitation. An equal volume of 20% TCA is added to the cell supernatant, incubated on ice for 1 hour and pelleted by centrifugation for 5 minutes. Pellets are washed in acetone, dried and resuspended in water. Cellular protein extracts are run on a 10% Bis-Tris NuPage (nuclear extracts) or 4-12% Tris- 20 Glycine (supernatant extracts) polyacrylamide gel and transferred onto nitro-cellulose membranes. Non-specific binding can be blocked by incubation, for example, with 5% non-fat milk for 1 hour followed by primary antibody for 16 hour at 4°C. Following washes, the secondary antibody is applied, for example (1:10,000 dilution) for 1 hour at room temperature and the signal detected with SuperSignal reagent (Pierce).

#### 25 Example 8: Models useful to evaluate the down-regulation of BCR-ABL gene expression

##### *Cell Culture*

There are numerous cell culture systems that can be used to analyze reduction of BCR-ABL levels either directly or indirectly by measuring downstream effects. For example,

cultured human chronic myelogenous leukemic cells (e.g., K562, HUVEC or HeLa cells) can be used in cell culture experiments to assess the efficacy of nucleic acid molecules of the invention. As such, K562, HUVEC or HeLa cells treated with nucleic acid molecules of the invention (e.g., siNA) targeting BCR-ABL RNA would be expected to have decreased BCR-

- 5 ABL expression capacity compared to matched control nucleic acid molecules having a scrambled or inactive sequence. In a non-limiting example, human chronic myelogenous leukemic cells (K562, HUVEC or HeLas) are cultured and BCR-ABL expression is quantified, for example by time-resolved immunofluorometric assay. BCR-ABL messenger-RNA expression is quantitated with RT-PCR in cultured K562, HUVEC or HeLas.
- 10 Untreated cells are compared to cells treated with siNA molecules transfected with a suitable reagent, for example a cationic lipid such as lipofectamine, and BCR-ABL protein and RNA levels are quantitated. Dose response assays are then performed to establish dose dependent inhibition of BCR-ABL expression. In another non-limiting example, cell culture experiments are carried out as described by Wilda *et al.*, 2002, *Oncogene*, 21, 5716.

- 15 In several cell culture systems, cationic lipids have been shown to enhance the bioavailability of oligonucleotides to cells in culture (Bennet, *et al.*, 1992, *Mol. Pharmacology*, 41, 1023-1033). In one embodiment, siNA molecules of the invention are complexed with cationic lipids for cell culture experiments. siNA and cationic lipid mixtures are prepared in serum-free DMEM immediately prior to addition to the cells. DMEM plus
- 20 additives are warmed to room temperature (about 20-25°C) and cationic lipid is added to the final desired concentration and the solution is vortexed briefly. siNA molecules are added to the final desired concentration and the solution is again vortexed briefly and incubated for 10 minutes at room temperature. In dose response experiments, the RNA/lipid complex is serially diluted into DMEM following the 10 minute incubation.

## 25 *Animal Models*

Evaluating the efficacy of anti-BCR-ABL agents in animal models is an important prerequisite to human clinical trials. A BCR-ABL transgenic mouse model has been described (Huettnner *et al.*, 2000, *Nature Genetics*, 24, 57-60) Four BCR-ABL1 transresponder lines (2, 3, 4 and 27) were established from founder animals. Transgenic mice



were born with the expected mendelian frequency and developed normally, indicating that the tetracycline-responsive expression system corrects for BCR-ABL1 toxicity in embryonic tissue. No mice transgenic for the transresponder construct developed any haematological disorder with a median follow-up period of 10 months. Double transgenic mice (BCR-  
5 ABL1-tetracycline transactivator (tTA)) were generated by breeding female transresponder mice with male mouse mammary tumour virus (MMTV)-tTA transactivator mice under continuous administration of tetracycline (0.5 g/l) in the drinking water, starting five days before mating. The genotypic distribution of double transgenic mice followed the predicted mendelian frequency in all four lines. Withdrawal of tetracycline administration in double  
10 transgenic animals allowed expression of BCR-ABL1 and resulted in the development of lethal leukemia in 100% of the mice within a time frame that was consistent within each line. Such transgenic mice are useful as models for cancer and for identifying nucleic acid molecules of the invention that modulate BCR-ABL gene expression and gene function toward the development of a therapeutic for use in treating cancer.

15 Example 9: Models useful to evaluate the down-regulation of ERG gene expression

*Cell Culture*

There are several cell-culture models that can be utilized to determine the efficacy of nucleic acid molecules of the instant invention directed against Erg expression. Hart *et al.*, 1995, *Oncogene*, 10(7), 1423-30, describe the transfection of NIH3T3 cells with an Erg  
20 expression construct consisting of human Erg cDNA driven by the sheep metallothionein 1a promoter (sMTERG). Established clonal cell lines overexpressing Erg became morphologically altered, grew in low-serum and serum free media, and gave rise to colonies in soft agar suspension. These colonies resulted in the formation of solid tumors when injected into nude mice. Yi *et al.*, 1997, *Oncogene*, 14(11), 1259-1268, describe the  
25 expression of Erg and aberrant Erg fusion proteins as inhibitory in the induction of apoptosis in NIH3T3 and Ewing's sarcoma cells induced by either serum deprivation or by treatment with calcium ionophore. Inhibition of the expression of the aberrant fusion proteins by antisense RNA techniques resulted in the increased susceptibility of these cells to apoptosis leading to cell death. As such, these cell lines can be used for the evaluation of nucleic acid

molecules of the instant invention via Erg RNA knockdown, Erg protein knockdown, and proliferation-based endpoints.

#### *Animal Models*

There are several animal models in which the anti-proliferative and anti-angiogenic effect of nucleic acids of the present invention, such as siRNA, directed against Erg RNA can be tested. The mouse model described by Hart *et al.*, *supra*, can be used to evaluate nucleic acid molecules of the instant invention *in vivo* for anti-tumorigenic capacity. Additional models can be used to study the anti-angiogenic capacity of the nucleic acid molecules of the instant invention. Typically a corneal model has been used to study angiogenesis in rat and rabbit since recruitment of vessels can easily be followed in this normally avascular tissue (Pandey *et al.*, 1995 *Science* 268: 567-569). In these models, a small Teflon or Hydrion disk pretreated with an angiogenic compound is inserted into a pocket surgically created in the cornea. Angiogenesis is monitored 3 to 5 days later. siRNA directed against ARNT, Tie-2 or integrin subunit RNAs would be delivered in the disk as well, or dropwise to the eye over the time course of the experiment. In another eye model, hypoxia has been shown to cause both increased expression of VEGF and neovascularization in the retina (Pierce *et al.*, 1995 *Proc. Natl. Acad. Sci. USA.* 92: 905-909; Shweiki *et al.*, 1992 *J. Clin. Invest.* 91: 2235-2243).

Another animal model that addresses neovascularization involves Matrigel, an extract of basement membrane that becomes a solid gel when injected subcutaneously (Passaniti *et al.*, 1992 *Lab. Invest.* 67: 519-528). When the Matrigel is supplemented with angiogenesis factors, vessels grow into the Matrigel over a period of 3 to 5 days and angiogenesis can be assessed. Again, siRNA directed against ARNT, Tie-2 or integrin subunit RNAs would be delivered in the Matrigel.

Several animal models exist for screening of anti-angiogenic agents. These include corneal vessel formation following corneal injury (Burger *et al.*, 1985 *Cornea* 4: 35-41; Lepri, *et al.*, 1994 *J. Ocular Pharmacol.* 10: 273-280; Ormerod *et al.*, 1990 *Am. J. Pathol.* 137: 1243-1252) or intracorneal growth factor implant (Grant *et al.*, 1993 *Diabetologia* 36:

282-291; Pandey *et al.* 1995 *supra*; Zieche *et al.*, 1992 *Lab. Invest.* 67: 711-715), vessel growth into Matrigel matrix containing growth factors (Passaniti *et al.*, 1992 *supra*), female reproductive organ neovascularization following hormonal manipulation (Shweiki *et al.*, 1993 *Clin. Invest.* 91: 2235-2243), several models involving inhibition of tumor growth in  
5 highly vascularized solid tumors (O'Reilly *et al.*, 1994 *Cell* 79: 315-328; Senger *et al.*, 1993 *Cancer and Metas. Rev.* 12: 303-324; Takahasi *et al.*, 1994 *Cancer Res.* 54: 4233-4237; Kim *et al.*, 1993 *supra*), and transient hypoxia-induced neovascularization in the mouse retina (Pierce *et al.*, 1995 *Proc. Natl. Acad. Sci. USA.* 92: 905-909).

The cornea model, described in Pandey *et al. supra*, is the most common and well  
10 characterized anti-angiogenic agent efficacy screening model. This model involves an avascular tissue into which vessels are recruited by a stimulating agent (growth factor, thermal or alkali burn, endotoxin). The corneal model would utilize the intrastromal corneal implantation of a Teflon pellet soaked in an angiogenic compound-Hydron solution to recruit blood vessels toward the pellet which can be quantitated using standard microscopic and  
15 image analysis techniques. To evaluate their anti-angiogenic efficacy, siRNA is applied topically to the eye or bound within Hydron on the Teflon pellet itself. This avascular cornea as well as the Matrigel (see below) provide for low background assays. While the corneal model has been performed extensively in the rabbit, studies in the rat have also been conducted.

20 The mouse model (Passaniti *et al.*, *supra*) is a non-tissue model which utilizes Matrigel, an extract of basement membrane (Kleinman *et al.*, 1986) or Millipore® filter disk, which can be impregnated with growth factors and anti-angiogenic agents in a liquid form prior to injection. Upon subcutaneous administration at body temperature, the Matrigel or Millipore® filter disk forms a solid implant. An angiogenic compound would be embedded  
25 in the Matrigel or Millipore® filter disk which would be used to recruit vessels within the matrix of the Matrigel or Millipore® filter disk that can be processed histologically for endothelial cell specific vWF (factor VIII antigen) immunohistochemistry, Trichrome-Masson stain, or hemoglobin content. Like the cornea, the Matrigel or Millipore® filter disk

are avascular; however, it is not tissue. In the Matrigel or Millipore® filter disk model, siRNA is administered within the matrix of the Matrigel or Millipore® filter disk to test their anti-angiogenic efficacy. Thus, delivery issues in this model, as with delivery of siRNA by Hydron- coated Teflon pellets in the rat cornea model, can be less problematic due to the  
5 homogeneous presence of the siRNA within the respective matrix.

Other model systems to study tumor angiogenesis is reviewed by Folkman, 1985 *Adv. Cancer. Res.*, 43, 175.

#### *Use of murine models*

For a typical systemic study involving 10 mice (20 g each) per dose group, 5 doses (1,  
10 3, 10, 30 and 100 mg/kg daily over 14 days continuous administration), approximately 400 mg of siRNA, formulated in saline would be used. A similar study in young adult rats (200 g) would require over 4 g. Parallel pharmacokinetic studies can involve the use of similar quantities of siRNA further justifying the use of murine models.

#### *siRNA and Lewis lung carcinoma and B-16 melanoma murine models*

15 Identifying a common animal model for systemic efficacy testing of siRNA is an efficient way of screening siRNA for systemic efficacy. The Lewis lung carcinoma and B-16 murine melanoma models are well accepted models of primary and metastatic cancer and are used for initial screening of anti-cancer. These murine models are not dependent upon the use of immunodeficient mice, are relatively inexpensive, and minimize housing concerns.  
20 Both the Lewis lung and B-16 melanoma models involve subcutaneous implantation of approximately  $10^6$  tumor cells from metastatically aggressive tumor cell lines (Lewis lung lines 3LL or D122, LLc-LN7; B-16-BL6 melanoma) in C57BL/6J mice. Alternatively, the Lewis lung model can be produced by the surgical implantation of tumor spheres (approximately 0.8 mm in diameter). Metastasis also can be modeled by injecting the tumor  
25 cells directly *i.v.*. In the Lewis lung model, microscopic metastases can be observed approximately 14 days following implantation with quantifiable macroscopic metastatic tumors developing within 21-25 days. The B-16 melanoma exhibits a similar time course

with tumor neovascularization beginning 4 days following implantation. Since both primary and metastatic tumors exist in these models after 21-25 days in the same animal, multiple measurements can be taken as indices of efficacy. Primary tumor volume and growth latency as well as the number of micro- and macroscopic metastatic lung foci or number of animals  
5 exhibiting metastases can be quantitated. The percent increase in lifespan can also be measured. Thus, these models would provide suitable primary efficacy assays for screening systemically administered siRNA formulations.

In the Lewis lung and B-16 melanoma models, systemic pharmacotherapy with a wide variety of agents usually begins 1-7 days following tumor implantation/inoculation with  
10 either continuous or multiple administration regimens. Concurrent pharmacokinetic studies can be performed to determine whether sufficient tissue levels of siRNA can be achieved for pharmacodynamic effect to be expected. Furthermore, primary tumors and secondary lung metastases can be removed and subjected to a variety of *in vitro* studies (*i.e.* target RNA reduction).

15 Delivery of siRNA and siRNA formulations in the Lewis lung model

Several siRNA formulations, including cationic lipid complexes which can be useful for inflammatory diseases (*e.g.* DIMRIE/DOPE, *etc.*) and RES evading liposomes which can be used to enhance vascular exposure of the siRNA, are of interest in cancer models due to their presumed biodistribution to the lung. Thus, liposome formulations can be used for  
20 delivering siRNA to sites of pathology linked to an angiogenic response.

Example 10: RNAi mediated inhibition of BCR-ABL and/or ERG RNA expression

siNA constructs (Table III) are tested for efficacy in reducing BCR-ABL and/or ERG RNA expression in, for example, K562, HUVEC or HeLa cells. Cells are plated approximately 24h before transfection in 96-well plates at 5,000-7,500 cells/well, 100  
25  $\mu$ l/well, such that at the time of transfection cells are 70-90% confluent. For transfection, annealed siNAs are mixed with the transfection reagent (Lipofectamine 2000, Invitrogen) in a volume of 50  $\mu$ l/well and incubated for 20 min. at room temperature. The siNA transfection mixtures are added to cells to give a final siNA concentration of 25 nM in a

volume of 150  $\mu$ l. Each siNA transfection mixture is added to 3 wells for triplicate siNA treatments. Cells are incubated at 37° for 24h in the continued presence of the siNA transfection mixture. At 24h, RNA is prepared from each well of treated cells. The supernatants with the transfection mixtures are first removed and discarded, then the cells are  
5 lysed and RNA prepared from each well. Target gene expression following treatment is evaluated by RT-PCR for the target gene and for a control gene (36B4, an RNA polymerase subunit) for normalization. The triplicate data is averaged and the standard deviations determined for each treatment. Normalized data are graphed and the percent reduction of target mRNA by active siNAs in comparison to their respective inverted control siNAs is  
10 determined.

In a non-limiting example, a siNA construct comprising ribonucleotides and 3'-terminal dithymidine caps is assayed along with a chemically modified siNA construct comprising 2'-deoxy-2'-fluoro pyrimidine nucleotides and purine ribonucleotides in which the sense strand of the siNA is further modified with 5' and 3'-terminal inverted deoxyabasic  
15 caps and the antisense strand comprises a 3'-terminal phosphorothioate internucleotide linkage. Additional stabilization chemistries as described in Table IV are similarly assayed for activity. These siNA constructs are compared to appropriate matched chemistry inverted controls. In addition, the siNA constructs are also compared to untreated cells, cells transfected with lipid and scrambled siNA constructs, and cells transfected with lipid alone  
20 (transfection control).

#### Example 11: RNAi mediated inhibition of ERG2 RNA expression

siNA constructs (Table I) are tested for efficacy in reducing ERG2 RNA expression in, for example in DLD1 cells. Cells are plated approximately 24h before transfection in 96-well plates at 5,000-7,500 cells/well, 100  $\mu$ l/well, such that at the time of transfection cells are 70-  
25 90% confluent. For transfection, annealed siNAs are mixed with the transfection reagent (Lipofectamine 2000, Invitrogen) in a volume of 50  $\mu$ l/well and incubated for 20 min. at room temperature. The siNA transfection mixtures are added to cells to give a final siNA concentration of 25 nM in a volume of 150  $\mu$ l. Each siNA transfection mixture is added to 3 wells for triplicate siNA treatments. Cells are incubated at 37° for 24h in the continued

presence of the siNA transfection mixture. At 24h, RNA is prepared from each well of treated cells. The supernatants with the transfection mixtures are first removed and discarded, then the cells are lysed and RNA prepared from each well. Target gene expression following treatment is evaluated by RT-PCR for the target gene and for a control gene (36B4, an RNA polymerase subunit) for normalization. The triplicate data is averaged and the standard deviations determined for each treatment. Normalized data are graphed and the percent reduction of target mRNA by active siNAs in comparison to their respective inverted control siNAs was determined.

In a non-limiting example, siNA constructs were screened for activity (see Figure 13) and compared to untreated cells, scrambled siNA control constructs (Scram1 and Scram2), and cells transfected with lipid alone (transfection control). As shown in Figure 13, the siNA constructs significantly reduce ERG2 RNA expression. Leads generated from such a screen are then further assayed. In a non-limiting example, siNA constructs comprising ribonucleotides and 3'-terminal dithymidine caps are assayed along with a chemically modified siNA construct comprising 2'-deoxy-2'-fluoro pyrimidine nucleotides and purine ribonucleotides, in which the sense strand of the siNA is further modified with 5' and 3'-terminal inverted deoxyabasic caps and the antisense strand comprises a 3'-terminal phosphorothioate internucleotide linkage. Additional stabilization chemistries as described in Table IV are similarly assayed for activity. These siNA constructs are compared to appropriate matched chemistry inverted controls. In addition, the siNA constructs are also compared to untreated cells, cells transfected with lipid and scrambled siNA constructs, and cells transfected with lipid alone (transfection control). Additional stabilization chemistries as described in Table IV are similarly assayed for activity.

#### Example 12: Indications

The present body of knowledge in BCR-ABL research indicates the need for methods to assay BCR-ABL activity and for compounds that can regulate BCR-ABL expression for research, diagnostic, and therapeutic use. As described herein, the nucleic acid molecules of the present invention can be used in assays to diagnose disease state related of BCR-ABL

levels. In addition, the nucleic acid molecules can be used to treat disease state related to BCR-ABL levels.

Particular conditions and disease states that can be associated with BCR-ABL expression modulation include including cancer (e.g. leukemia, such as CML) and any other  
5 indications that can respond to the level of BCR-ABL in a cell or tissue.

Particular conditions and disease states that can be associated with ERG expression modulation include but are not limited to a broad spectrum of oncology and neovascularization-related indications, including but not limited to cancers of the lung, colon, breast, prostate, and cervix, lymphoma, Ewing's sarcoma and related tumors, melanoma,  
10 angiogenic disease states such as tumor angiogenesis, diabetic retinopathy, macular degeneration, neovascular glaucoma, myopic degeneration, arthritis such as rheumatoid arthritis, psoriasis, verruca vulgaris, angiofibroma of tuberous sclerosis, port-wine stains, Sturge Weber syndrome, Kippel-Trenaunay-Weber syndrome, Osler-Weber-rendu syndrome, leukemias such as acute myeloid leukemia, osteoporosis, wound healing and any other  
15 diseases or conditions that are related to or will respond to the levels of ERG in a cell or tissue, alone or in combination with other therapies.

Immunomodulators and chemotherapeutics are non-limiting examples of pharmaceutical agents that can be combined with or used in conjunction with the nucleic acid molecules (e.g. siNA molecules) of the instant invention. The use of radiation treatments and  
20 chemotherapeutics, such as Gemcytabine and cyclophosphamide, are non-limiting examples of chemotherapeutic agents that can be combined with or used in conjunction with the nucleic acid molecules (e.g. siNA molecules) of the instant invention. Those skilled in the art will recognize that other anti-cancer compounds and therapies can similarly be readily combined with the nucleic acid molecules of the instant invention (e.g. siNA molecules) and  
25 are hence within the scope of the instant invention. Such compounds and therapies are well known in the art (see for example *Cancer: Principles and Pranclice of Oncology*, Volumes 1 and 2, eds Devita, V.T., Hellman, S., and Rosenberg, S.A., J.B. Lippincott Company, Philadelphia, USA; incorporated herein by reference) and include, without limitation, folates, antifolates, pyrimidine analogs, fluoropyrimidines, purine analogs, adenosine analogs,



topoisomerase I inhibitors, anthracyclins, retinoids, antibiotics, anthacyclins, platinum analogs, alkylating agents, nitrosoureas, plant derived compounds such as vinca alkaloids, epipodophyllotoxins, tyrosine kinase inhibitors, taxols, radiation therapy, surgery, nutritional supplements, gene therapy, radiotherapy, for example 3D-CRT, immunotoxin therapy, for example ricin, and monoclonal antibodies. Specific examples of chemotherapeutic compounds that can be combined with or used in conjunction with the nucleic acid molecules of the invention include, but are not limited to, Paclitaxel; Docetaxel; Methotrexate; Doxorubin; Edatrexate; Vinorelbine; Tomaxifen; Leucovorin; 5-fluoro uridine (5-FU); Ionotecan; Cisplatin; Carboplatin; Amsacrine; Cytarabine; Bleomycin; Mitomycin C; Dactinomycin; Mithramycin; Hexamethylmelamine; Dacarbazine; L-asparaginase; Nitrogen mustard; Melphalan, Chlorambucil; Busulfan; Ifosfamide; 4-hydroperoxycyclophosphamide; Thiotepa; Irinotecan (CAMPTOSAR®, CPT-11, Camptothecin-11, Campto) Tamoxifen; Herceptin; IMC C225; ABX-EGF; and combinations thereof. The above list of compounds are non-limiting examples of compounds and/or methods that can be combined with or used in conjunction with the nucleic acid molecules (e.g. siNA) of the instant invention. Those skilled in the art will recognize that other drug compounds and therapies can similarly be readily combined with the nucleic acid molecules of the instant invention (e.g., siNA molecules) are hence within the scope of the instant invention.

#### Example 13: Diagnostic uses

The siNA molecules of the invention can be used in a variety of diagnostic applications, such as in the identification of molecular targets (e.g., RNA) in a variety of applications, for example, in clinical, industrial, environmental, agricultural and/or research settings. Such diagnostic use of siNA molecules involves utilizing reconstituted RNAi systems, for example, using cellular lysates or partially purified cellular lysates. siNA molecules of this invention can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of endogenous or exogenous, for example viral, RNA in a cell. The close relationship between siNA activity and the structure of the target RNA allows the detection of mutations in any region of the molecule, which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple siNA molecules

described in this invention, one can map nucleotide changes, which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with siNA molecules can be used to inhibit gene expression and define the role of specified gene products in the progression of disease or infection. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes, siNA molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations siNA molecules and/or other chemical or biological molecules). Other *in vitro* uses of siNA molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with a disease, infection, or related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a siNA using standard methodologies, for example, fluorescence resonance emission transfer (FRET).

In a specific example, siNA molecules that cleave only wild-type or mutant forms of the target RNA are used for the assay. The first siNA molecules (*i.e.*, those that cleave only wild-type forms of target RNA) are used to identify wild-type RNA present in the sample and the second siNA molecules (*i.e.*, those that cleave only mutant forms of target RNA) are used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both siNA molecules to demonstrate the relative siNA efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus, each analysis requires two siNA molecules, two substrates and one unknown sample, which is combined into six reactions. The presence of cleavage products is determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, disease related or infection related) is adequate to establish risk. If probes of comparable specific activity are used for both

transcripts, then a qualitative comparison of RNA levels is adequate and decreases the cost of the initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

5 All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

10 One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

15 It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims. The present invention teaches one skilled in the art to test various combinations and/or substitutions of chemical modifications described  
20 herein toward generating nucleic acid constructs with improved activity for mediating RNAi activity. Such improved activity can comprise improved stability, improved bioavailability, and/or improved activation of cellular responses mediating RNAi. Therefore, the specific embodiments described herein are not limiting and one skilled in the art can readily appreciate that specific combinations of the modifications described herein can be tested  
25 without undue experimentation toward identifying siNA molecules with improved RNAi activity.

The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein.

Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding  
5 any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and  
10 variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of  
15 members of the Markush group or other group.

Table I: BCR-ABL and ERG Accession Numbers

NM_004327	Homo sapiens breakpoint cluster region (BCR), transcript variant 1, mRNA gi 11038638 ref NM_004327.2 [11038638]
NM_021574	Homo sapiens breakpoint cluster region (BCR), transcript variant 2, mRNA gi 11038640 ref NM_021574.1 [11038640]
NM_005157	Homo sapiens v-abl Abelson murine leukemia viral oncogene homolog 1 (ABL1), transcript variant a, mRNA gi 6382056 ref NM_005157.2 [6382056]
NM_007313	Homo sapiens v-abl Abelson murine leukemia viral oncogene homolog 1 (ABL1), transcript variant b, mRNA gi 6382057 ref NM_007313.1 [6382057]
AJ131467	Homo sapiens mRNA for BCR/ABL chimeric fusion peptide, partial gi 4033556 emb AJ131467.1 HSA131467[4033556]
AJ131466	Homo sapiens mRNA for BCR/ABL (major breakpoint) fusion peptide, partial gi 4033554 emb AJ131466.1 HSA131466[4033554]
AF044317	Homo sapiens TEL/AML1 fusion gene, partial sequence gi 2920622 gb AF044317.1 AF044317[2920622]

AF327066  
Homo sapiens Ewings sarcoma EWS-Flil (type 1) oncogene mRNA, complete cds  
gi|12963354|gb|AF327066.1|AF327066[12963354].

S71805  
TLS/FUS...ERG {translocation} [human, myeloid leukemia patient, peripheral  
blood, bone marrow cells, mRNA Partial Mutant, 3 genes, 99 nt]  
gi|560579|bbm|344598|bbs|151117|gb|S71805.1|S71805[560579]

AF178854  
Synthetic construct Pax3-forkhead fusion protein (Pax3/FKHR) mRNA, complete cds  
gi|6636096|gb|AF178854.1|AF178854[6636096]

S78159  
Homo sapiens AML1-ETO fusion protein (AML1-ETO) mRNA, partial cds  
gi|999360|bbm|371144|bbs|166913|gb|S78159.1|S78159[999360]

NM\_004449  
Homo sapiens v-ets erythroblastosis virus E26 oncogene like (avian) (ERG), mRNA  
gi|7657065|ref|NM\_004449.2|[7657065]

M21535  
Human erg protein (ets-related gene) mRNA, complete cds  
gi|182182|gb|M21535.1|HUMERG11[182182]

M21536  
Human erg protein (ets-related gene) mRNA, 3' flank  
gi|182183|gb|M21536.1|HUMERG12[182183]

M21535  
Human erg protein (ets-related gene) mRNA, complete cds  
gi|182182|gb|M21535.1|HUMERG11[182182]

M98833  
Homo sapiens ERGB transcription factor mRNA, complete cds  
gi|7025922|gb|M98833.3|HUMERGBFLI[7025922]

X67001  
H.sapiens HUMFLI-1 mRNA  
gi|32529|emb|X67001.1|HSHUMFLI[32529]

M93255  
Human FLI-1 mRNA, complete cds for two alternate splicings  
gi|182659|gb|M93255.1|HUMFLI1A[182659]

NM\_002017  
Homo sapiens Friend leukemia virus integration 1 (FLI1), mRNA  
gi|7110592|ref|NM\_002017.2|[7110592]

S45205  
Fli-1=Friend leukemia integration 1 [human, mRNA, 1673 nt]  
gi|257353|bbm|246089|bbs|115336|gb|S45205.1|S45205[257353]

S45205  
GI number 628772 references a Protein record; you are currently using the  
Nucleotide database.

S82338  
Homo sapiens fusion gene (ERG/EWS) gene, partial cds  
gi|1703711|bbm|387740|bbs|178240|gb|S82338.1|S82338[1703711]

S82335  
EWS/ERG=fusion gene {EWS exon 7 - ERG exon 8, translocation} [human, left iliac  
bone, liver, osteolytic tumor patient, MON isolate, Genomic, 74 nt]  
gi|1703709|bbm|387732|bbs|178239|gb|S82335.1|S82335[1703709]

## S73762

EWS...erg {reciprocal translocation junction site} [human, Ewing's sarcoma cell line #5838 cells, Genomic Mutant, 3 genes, 267 nt]  
gi|688241|bbm|352440|bbs|156728|gb|S73762.1|S73762[688241]

## S73762

GI number 2146518 references a Protein record; you are currently using the Nucleotide database.

## S72865

EWS...EWS-erg=EWS-erg fusion protein type 9e [human, SK-PN-LI cell line, mRNA Partial Mutant, 3 genes, 588 nt]  
gi|633777|bbm|347812|bbs|154042|gb|S72865.1|S72865[633777]

## S72865

GI number 2145741 references a Protein record; you are currently using the Nucleotide database.

## S72622

EWS-erg=EWS-erg fusion protein type 3e {translocation, type 3e} [human, T92-60 tumor, mRNA Partial Mutant, 54 nt]  
gi|633775|bbm|347423|bbs|153611|gb|S72622.1|S72622[633775]

## S72621

EWS...erg {translocation, type 1e and 9e} [human, SK-PN-LI cell line, mRNA Partial Mutant, 3 genes, 762 nt]  
gi|633773|bbm|347409|bbs|153609|gb|S72621.1|S72621[633773]

## S70593

Homo sapiens EWS/ERG fusion protein (EWS/ERG) mRNA, partial cds  
gi|546447|bbm|340883|bbs|148946|gb|S70593.1|S70593[546447]

## S70579



Homo sapiens EWS/ERG fusion protein (EWS/ERG) mRNA, partial cds  
gi|546445|bbm|340872|bbs|148944|gb|S70579.1|S70579[546445]

AB028209  
Mus musculus mRNA, up-regulated by FUS-ERG, 3' region, cDNA fragment: C14G220  
gi|6139005|dbj|AB028209.1|[6139005]

Y10001  
H.sapiens DNA fragment containing fusion point of FUS gene and ERG gene,  
translocation t(16;21)(p11;q22)  
gi|2181922|emb|Y10001.1|HSY10001[2181922]

S77574  
TLS...ERG {translocation} [human, acute non-lymphocytic leukemia cell lines  
IRTA17 and IRTA21, mRNA Partial, 3 genes, 211 nt]  
gi|957350|bbm|369615|bbs|165809|gb|S77574.1|S77574[957350]

Table II: BCR-ABL and ERG siNA and Target Sequences

NM\_004327 (BCR)

Pos	Target Sequence	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	GGAGUAAGGUAAGGUAAGC	1	3	GGAGUAAGGUAAGGUAAGC	1	21	GCUACUCCUACCUAUCUCC	264
21	CGUGUAAGGGCGAUGAGU	2	21	CGUGUAAGGGCGAUGAGU	2	39	ACUACUCCUACCUAUCUCC	265
39	UGUGGGCGGGCGGAGUG	3	39	UGUGGGCGGGCGGAGUG	3	57	CACUCCCGCCGCGCCACAC	266
57	GCGCAGAGCGGCGUGGC	4	57	GCGCAGAGCGGCGUGGC	4	75	GCCAGCCGCGCUCGCGCGC	267
75	CUGAGCUUAGCGUCCGAGG	5	75	CUGAGCUUAGCGUCCGAGG	5	93	CCUCCGACGCUAAGCUCAG	268
93	GAGCGGGCGGGCGGCGGG	6	93	GAGCGGGCGGGCGGCGGG	6	111	CCGCCGCGCGCGCGCGCUC	269
111	GCGGAGCGGGCGGCGGG	7	111	GCGGAGCGGGCGGCGGG	7	129	CCGCCGCGCGCGCGCGCGC	270
129	GGGCUUGGGCGGUGCGG	8	129	GGGCUUGGGCGGUGCGG	8	147	CCGCACCGCCCGACAGCCC	271
147	GAAGCGAGAGCGGAGAGC	9	147	GAAGCGAGAGCGGAGAGC	9	165	GCUCUCCGCGCUCGCGCUC	272
165	CGCGCGGCGGUGGCCAGA	10	165	CGCGCGGCGGUGGCCAGA	10	183	UCUGGCCACGCGCCCGCGCG	273
183	AGUCUGGCGGCGGCGUGGC	11	183	AGUCUGGCGGCGGCGUGGC	11	201	GCCAGGCGCGCGCGCGACU	274
201	CGGAGCGGAGAGCAGCGCC	12	201	CGGAGCGGAGAGCAGCGCC	12	219	GGCGCUCGUCUCCGCGCUC	275
219	CCGCGCCUCCGCGUGCGGA	13	219	CCGCGCCUCCGCGUGCGGA	13	237	UCCGCACGGCGAGCGCGCG	276
237	AGGAGCCCGCACACAUA	14	237	AGGAGCCCGCACACAUA	14	255	UAUUGUGUGCGGGCGCUCU	277
255	AGCGCGCGCGCAGCCCGC	15	255	AGCGCGCGCGCAGCCCGC	15	273	GCGGGCUCGCGCGCGCGCUC	278
273	CGCCUUCGCGCGCGCGCG	16	273	CGCCUUCGCGCGCGCGCG	16	291	CGCGCGGGGGGAAAGCGCG	279
291	GCGCGCGCGCGCGCGCG	17	291	GCGCGCGCGCGCGCGCG	17	309	UCGGCGCGGGGGCGGGCG	280
309	AGCGCGCGCGCGCGCGCUA	18	309	AGCGCGCGCGCGCGCGCUA	18	327	UGAGGCGGAGCGGGCGCGCU	281
327	ACCGCGCAGCGGAGUGG	19	327	ACCGCGCAGCGGAGUGG	19	345	CCACUCCUUGGUGGCGAGGU	282
345	GCGCGCAUUGUCGCGCG	20	345	GCGCGCAUUGUCGCGCG	20	363	GCGGCGAACAAUUGCCCGCC	283
363	CGCGCGCGCGCGCGCGGG	21	363	CGCGCGCGCGCGCGCGGG	21	381	CCCCGCGCGCGCGCGCGCG	284
381	GCCAUUGGGGCGCGCGCG	22	381	GCCAUUGGGGCGCGCGCG	22	399	GCCGGGCGCGCGCGCGCG	285
399	CGCGCGCGCGCGCGCGCG	23	399	CGCGCGCGCGCGCGCGCG	23	417	CCAGGCGCGCGCGCGCGCG	286
417	GCGAGCGCGCGCGCGCGCG	24	417	GCGAGCGCGCGCGCGCGCG	24	435	GGCGCGCGCGCGCGCGCG	287
435	GCGAGAGCGCGCGCGCGCG	25	435	GCGAGAGCGCGCGCGCGCG	25	453	GCGGGGCGCGCGCGCGCG	288
453	GCGAGCGCGCGCGCGCGCG	26	453	GCGAGCGCGCGCGCGCGCG	26	471	CUGCGCGCGCGCGCGCGCG	289
471	GGUAAAGCGCGCGCGCGCA	27	471	GGUAAAGCGCGCGCGCGCA	27	489	UGGCGCGCGCGCGCGCGCG	290
489	AUGGUGGACCGGUGGGCU	28	489	AUGGUGGACCGGUGGGCU	28	507	AGCCACCGCGGCGCGCGCG	291
507	UUCGCGGAGCGGUGGAAGG	29	507	UUCGCGGAGCGGUGGAAGG	29	525	CCUCCACGCGCGCGCGCG	292
525	GCGCAGUUCGCGGACUCAG	30	525	GCGCAGUUCGCGGACUCAG	30	543	CUGAGUCGCGGGAACUGCGC	293
543	GAGCCCCGCGCGGAGGAGC	31	543	GAGCCCCGCGCGGAGGAGC	31	561	GCUCCAUUGCGCGGGGCGUC	294
561	CUGCGCUCAGUGGCGGACA	32	561	CUGCGCUCAGUGGCGGACA	32	579	UGUCGCGCGCGCGCGCGCG	295

579	AUCGAGCAGGAGCUGGAGC	33	579	AUCGAGCAGGAGCUGGAGC	33	597	GCUCAGCUCUCCUGCUGAU	296
597	CGCUGCAAGGCCUCCAUUC	34	597	CGCUGCAAGGCCUCCAUUC	34	615	GAUUGAGGCCUUGCAGCG	297
615	CGCGCCUUGGAGCAGGAGG	35	615	CGCGCCUUGGAGCAGGAGG	35	633	CCUCCUGCUCUCCAGGCGCG	298
633	GUGAACCCAGGAGCGCUUC	36	633	GUGAACCCAGGAGCGCUUC	36	651	GGAAGCGCUCUCCUGGUUAC	299
651	CGCAUGAUCUACCUUGCAGA	37	651	CGCAUGAUCUACCUUGCAGA	37	669	UCUGCAGGUAGAUCAUGCG	300
669	ACGUUGCUGGCCAAGGAAA	38	669	ACGUUGCUGGCCAAGGAAA	38	687	UUUCCUUGGCCAGCAACGU	301
687	AAGAAGAGCUAUGACCGGC	39	687	AAGAAGAGCUAUGACCGGC	39	705	GCCGGUCAUAGCUUUCUU	302
705	CAGCGAUGGGGCUUCCGGC	40	705	CAGCGAUGGGGCUUCCGGC	40	723	GCCGGAAGCCCCAUCGCG	303
723	CGCGCGCGCAGGCCCGCC	41	723	CGCGCGCGCAGGCCCGCC	41	741	CGGGGCCUUGCGCCGCGCG	304
741	GACGGCGCUCCGAGCCCG	42	741	GACGGCGCUCCGAGCCCG	42	759	GGGGCUCGGAGGCGCGGUC	305
759	CGAGCGUCCGCGUGCGCC	43	759	CGAGCGUCCGCGUGCGCC	43	777	GGCGCGACGCGGACGCUCG	306
777	CCGACGCCAGCGCCCGCG	44	777	CCGACGCCAGCGCCCGCG	44	795	CGCGGGCGCUGGCUGCGG	307
795	GACGAGCCGACCCGCGCG	45	795	GACGAGCCGACCCGCGCG	45	813	CGCGGGGUCGGCUCGCGUC	308
813	CCGCGCGAGGAGCCCGGAG	46	813	CCGCGCGAGGAGCCCGGAG	46	831	CCUCGGGCUCCUCGGCGGG	309
831	GCCCGGCCGACGCGGAGG	47	831	GCCCGGCCGACGCGGAGG	47	849	CCUCGGGCUCCUCGGCGGG	310
849	GGUUCUCCGGGUAAGGCCA	48	849	GGUUCUCCGGGUAAGGCCA	48	867	UGGCCUUAACCCGGGAGAAC	311
867	AGGCCGGGACCGCCGCGA	49	867	AGGCCGGGACCGCCGCGA	49	885	UGCGGGCGUCCCGGGGCU	312
885	AGGCCGGGACCGCCGCGU	50	885	AGGCCGGGACCGCCGCGU	50	903	ACGCGGCUCCCGCGGGCU	313
903	UCGGGGAAACGGGACGACC	51	903	UCGGGGAAACGGGACGACC	51	921	GGUCGUCCGUUCCCGCGA	314
921	CGGGACCCCGCCGACGCG	52	921	CGGGACCCCGCCGACGCG	52	939	CGCUGCGGGGGGUCGCCCG	315
939	GUGCGGCGCUCAGGUCCA	53	939	GUGCGGCGCUCAGGUCCA	53	957	UGGACCUAGAGCGCGGCCAC	316
957	AACUUCGAGCGGAUCCGCA	54	957	AACUUCGAGCGGAUCCGCA	54	975	UGCGGAUCCGCUCCGAAAGU	317
975	AAGGGCCAUUGCCAGCCCG	55	975	AAGGGCCAUUGCCAGCCCG	55	993	CGGGCUGGGCCAUGGCCCU	318
993	GGGGCGGACGCGGAGAAC	56	993	GGGGCGGACGCGGAGAAC	56	1011	GCUCUCGGCGUCCGCCCG	319
1011	CCUUCUACGUGAACGUCG	57	1011	CCUUCUACGUGAACGUCG	57	1029	CGACGUUACGUGAAGAGGG	320
1029	GAGUUCACACGAGCGCG	58	1029	GAGUUCACACGAGCGCG	58	1047	CGCGCUCGUGGUGAAACUC	321
1047	GGCCUGGUGAAGGUCAACG	59	1047	GGCCUGGUGAAGGUCAACG	59	1065	CGUUGACCUUACACAGGCG	322
1065	GACAAAGAGGUGUCGGACC	60	1065	GACAAAGAGGUGUCGGACC	60	1083	GGUCCGACACCUUUGUUC	323
1083	CGCAUCAGCUCUCCUGGCA	61	1083	CGCAUCAGCUCUCCUGGCA	61	1101	UGCCCAGGAGCUGAUUGCG	324
1101	AGCCAGGCCAUGCAGAUUG	62	1101	AGCCAGGCCAUGCAGAUUG	62	1119	CCAUCUGCAUGGCCUGGCU	325
1119	GAGCGCAAAAAGUCCGAGC	63	1119	GAGCGCAAAAAGUCCGAGC	63	1137	GCUGGGACUUAUUGCGCUC	326
1137	CACGGCGGGGCUUGAGCG	64	1137	CACGGCGGGGCUUGAGCG	64	1155	CGCUCGAGCCCGCGCGCGUG	327
1155	GUGGGGGAUGCAUCCAGCG	65	1155	GUGGGGGAUGCAUCCAGCG	65	1173	GCCUGGAUGCAUCCCGCAC	328
1173	CCCCUUAACCGGGGACGCU	66	1173	CCCCUUAACCGGGGACGCU	66	1191	AGCGUCCCGGUAAGGGGG	329
1191	UCCUCGAGAGCAGCUGCG	67	1191	UCCUCGAGAGCAGCUGCG	67	1209	CGCAGCUCGCUCCGAGGA	330
1209	GGCGUCGACGCGGACUACG	68	1209	GGCGUCGACGCGGACUACG	68	1227	CGUAGUCGCCCGCUCGACGCC	331

1227	GAGGACGCCGAGUUGAAC	69	1227	GAGGACGCCGAGUUGAAC	69	1245	GGUUAACUCGGCGUCCUC	332
1245	CCCCGUUCCUGAAGGACA	70	1245	CCCCGUUCCUGAAGGACA	70	1263	UGUCCUUCAGGAAGCGGG	333
1263	AACUGAUCGACGCCAAUG	71	1263	AACUGAUCGACGCCAAUG	71	1281	CAUUGGCGUCGACAGGUU	334
1281	GGCGUAGCAGGCCCCCUU	72	1281	GGCGUAGCAGGCCCCCUU	72	1299	AAGGGGCCUGCUACCGCC	335
1299	UGGCCGCCUGGAGUACC	73	1299	UGGCCGCCUGGAGUACC	73	1317	GGUACUCGAGGGCGGCCA	336
1317	CAGCCUACAGAGCAUCU	74	1317	CAGCCUACAGAGCAUCU	74	1335	AGAUCUCUGGAGGGCUG	337
1335	UACUCGGGGCAUGAUGG	75	1335	UACUCGGGGCAUGAUGG	75	1353	CCAUCAGCCCCCGACGUA	338
1353	GAAGGGAGGGCAAGGGCC	76	1353	GAAGGGAGGGCAAGGGCC	76	1371	GGCCUUGCCCCCUCCUUC	339
1371	CCGUCUCGCGCAGCCAGA	77	1371	CCGUCUCGCGCAGCCAGA	77	1389	UCUGGCGCGCAGGAGCGG	340
1389	AGCACUCUGAGCAGGAGA	78	1389	AGCACUCUGAGCAGGAGA	78	1407	UCUCCUGCUCAGAGGUCU	341
1407	AAGCGCCUACCGGCCCC	79	1407	AAGCGCCUACCGGCCCC	79	1425	GGGCCAGGUAAGGCGCUU	342
1425	CGCAGGUCCUACUCCCCC	80	1425	CGCAGGUCCUACUCCCCC	80	1443	GGGGGAGUAGGACCUJCG	343
1443	CGGAGUUGAGGAUJCG	81	1443	CGGAGUUGAGGAUJCG	81	1461	CGCAUCCUCAAACUCCG	344
1461	GGAGCGGCUAUACCCCG	82	1461	GGAGCGGCUAUACCCCG	82	1479	CCGGGUUAGCCGCCUCC	345
1479	GACUGCAGCUCAAUGAGA	83	1479	GACUGCAGCUCAAUGAGA	83	1497	UCUCAUUGGAGCUGCAGU	346
1497	AACUCACCUCCAGCGAGG	84	1497	AACUCACCUCCAGCGAGG	84	1515	CCUCGCUGGAGGUGAGGU	347
1515	GAGGACUUCUCCUGGCC	85	1515	GAGGACUUCUCCUGGCC	85	1533	GGCCAGAGGAGAAGUCCUC	348
1533	CAGUCCAGCGCGUJGCC	86	1533	CAGUCCAGCGCGUJGCC	86	1551	GGGACACGCGGCUUGGACUG	349
1551	CCAAGCCCAACCUUACC	87	1551	CCAAGCCCAACCUUACC	87	1569	GGUAGGUGGUGGGCUUUG	350
1569	CGCAUGUCCGGGACAAA	88	1569	CGCAUGUCCGGGACAAA	88	1587	UUUUUCCCCGGAACAUGCG	351
1587	AGCCGCUCCUCCGAGAGA	89	1587	AGCCGCUCCUCCGAGAGA	89	1605	UCUGCGAGGGAGAGCGGCU	352
1605	AACUCGCAACAGUCCUUCG	90	1605	AACUCGCAACAGUCCUUCG	90	1623	CGAAGGACUUGUCCGAGUU	353
1623	GACAGCAGCAGUCCCCCA	91	1623	GACAGCAGCAGUCCCCCA	91	1641	UGGGGGGACUGCUGCUGUC	354
1641	ACGCCGAGUGCCCAAGC	92	1641	ACGCCGAGUGCCCAAGC	92	1659	GCUUUAGGCACUGCGGCGU	355
1659	CGGCACCGGCACUGCCCG	93	1659	CGGCACCGGCACUGCCCG	93	1677	CCGGGACUGCCGGUGCCG	356
1677	GUUGUCGUGCCGAGGCCA	94	1677	GUUGUCGUGCCGAGGCCA	94	1695	UGGCCUCGGACACGACAAC	357
1695	ACCAUCGUGGGGUCGCGA	95	1695	ACCAUCGUGGGGUCGCGA	95	1713	UGCGGACGCCACACGALUGU	358
1713	AAGACCGGCAGAUUGGC	96	1713	AAGACCGGCAGAUUGGC	96	1731	GCCAGAUUCGCCCGGUCUU	359
1731	CCCAACGAUGGCGAGGGCG	97	1731	CCCAACGAUGGCGAGGGCG	97	1749	CGCCUCCGCCAUGGUUGGG	360
1749	GCCUUCGAUGGAGACGCAG	98	1749	GCCUUCGAUGGAGACGCAG	98	1767	CUGCGUCUCCAUUGGAAGGC	361
1767	GAUGGCUUGGGAACAC	99	1767	GAUGGCUUGGGAACAC	99	1785	GUGUCCGAACAGAGCCAUC	362
1785	CCACCUUGGAUAGGCGUGG	100	1785	CCACCUUGGAUAGGCGUGG	100	1803	CGCAGCCGUAUCCAGGUGG	363
1803	GCUGCAGACCGGCAGAGG	101	1803	GCUGCAGACCGGCAGAGG	101	1821	CCUCUGCCCGGUCUGCAGC	364
1821	GAGCAGCGCCGGCACCAG	102	1821	GAGCAGCGCCGGCACCAG	102	1839	CUUGGUGCCGGCGGCUCCUC	365
1839	GAUGGGCUGCCCUACAUUG	103	1839	GAUGGGCUGCCCUACAUUG	103	1857	CAUUGAGGGCAGCCCAUC	366
1857	GAUGACUCGCCCUCCUCAU	104	1857	GAUGACUCGCCCUCCUCAU	104	1875	AUGAGGAGGGCGAGUCAUC	367

1875	UCGCCCCACCCUCAGCAGCA	105	1875	UCGCCCCACCCUCAGCAGCA	105	1893	UGCUGCUGAGGUGGGGCGA	368
1883	AAGGGCAGGGGCGAGCCGGG	106	1893	AAGGGCAGGGGCGAGCCGGG	106	1911	CCCGGUGCCCCUGCCCCUU	369
1911	GAUGCGUGGUCUCGGGAG	107	1911	GAUGCGUGGUCUCGGGAG	107	1929	CUCCGAGACGACGCGCAUC	370
1929	GCCUGGAGUCCACUAAAG	108	1929	GCCUGGAGUCCACUAAAG	108	1947	CUUAGUGGACUCCAGGGC	371
1947	GCGAGUGAGCUGGACUUGG	109	1947	GCGAGUGAGCUGGACUUGG	109	1965	CCAAGUCCAGCUCACUCGC	372
1965	GAAAAGGCUUGGAGAUAGA	110	1965	GAAAAGGCUUGGAGAUAGA	110	1983	UCUUCUCCAGCCCUUUUC	373
1983	AGAAAUGGGUCCUGUCGG	111	1983	AGAAAUGGGUCCUGUCGG	111	2001	CCBACAGACCCCAUUUUUC	374
2001	GAAUCCUGGCUAGCGAGG	112	2001	GAAUCCUGGCUAGCGAGG	112	2019	CCUCGCUAGCCAGGAUUC	375
2019	GAGACUUAACCUAGGCCACC	113	2019	GAGACUUAACCUAGGCCACC	113	2037	GGUGGCUAGGUAAGUCUC	376
2037	CUGGAGGCACUGCUCUGC	114	2037	CUGGAGGCACUGCUCUGC	114	2055	GCAGCAGCAGUGCCUCCAG	377
2055	CCCAUGAAGCCUUUAGAAAG	115	2055	CCCAUGAAGCCUUUAGAAAG	115	2073	CUUCAAAGGCUUCAUGGG	378
2073	GCCGCUGCCACCCACCUUC	116	2073	GCCGCUGCCACCCACCUUC	116	2091	GAGAGGUGGUGGCGCGGC	379
2091	CAGCGGUGCUGACGAGUC	117	2091	CAGCGGUGCUGACGAGUC	117	2109	GACUCGUCAGCACCAGGUC	380
2109	CAGCAGUCCGAGACCAUCU	118	2109	CAGCAGUCCGAGACCAUCU	118	2127	AGAUGGUCUCGACUUGCUG	381
2127	UUCUCAAAGUGCCUGAGC	119	2127	UUCUCAAAGUGCCUGAGC	119	2145	GCUCAGGCACUUAAGAA	382
2145	CUCUACGAGAUCCACAAAG	120	2145	CUCUACGAGAUCCACAAAG	120	2163	CCUUGUGGAUCUCGUAGAG	383
2163	GAGUUCUUAUGGUGGUCUC	121	2163	GAGUUCUUAUGGUGGUCUC	121	2181	AGACCCCAUCAUAGAACUC	384
2181	UUCGCCCGCGUGCAGCAGU	122	2181	UUCGCCCGCGUGCAGCAGU	122	2199	ACUGCUCACGCGGGGAA	385
2199	UGGAGCCACCGAGCAGCGG	123	2199	UGGAGCCACCGAGCAGCGG	123	2217	CCCGCUGCUGGUGGCUCCA	386
2217	GUGGGCGACCCUUCUCCAGA	124	2217	GUGGGCGACCCUUCUCCAGA	124	2235	UCUGGAAGAGGUGCGCCAC	387
2235	AAGCUGGCCAGCCAGCUGG	125	2235	AAGCUGGCCAGCCAGCUGG	125	2253	CCAGCUGGCGUGGCCAGCUU	388
2253	GGUGUGUACCGGGCCUUCG	126	2253	GGUGUGUACCGGGCCUUCG	126	2271	CGAAGGCCCGGUAACACAC	389
2271	GUGGACAAACUACGGAGUUG	127	2271	GUGGACAAACUACGGAGUUG	127	2289	CAACUCCGUAUUGUCCAGC	390
2289	GCCAUUGGAAUUGGCUGAGA	128	2289	GCCAUUGGAAUUGGCUGAGA	128	2307	UCUCAGCCAUUCCAGUCC	391
2307	AAGUCUGUCAGGCCAAUUG	129	2307	AAGUCUGUCAGGCCAAUUG	129	2325	CAUUGGCCUGACAGCACUU	392
2325	GCUCAGUUGCAGAAUUCU	130	2325	GCUCAGUUGCAGAAUUCU	130	2343	AGAUUUCUGCAAACUGAGC	393
2343	UCCGAGAACCUAGAGGCCA	131	2343	UCCGAGAACCUAGAGGCCA	131	2361	UGGCUCUCAGGUUCUCGGA	394
2361	AGAAGCAACAAAGAUGCCA	132	2361	AGAAGCAACAAAGAUGCCA	132	2379	UGGCAUCUUGUUGUUCUUCU	395
2379	AAGGAUCCAAACGACCAAGA	133	2379	AAGGAUCCAAACGACCAAGA	133	2397	UCUUGGUCUGUUGGAUCCUU	396
2397	AACUCUCUGGAAACUCUGC	134	2397	AACUCUCUGGAAACUCUGC	134	2415	GCAGAGUUCAGAGAGUU	397
2415	CUCUACAAGCCUGUGGACC	135	2415	CUCUACAAGCCUGUGGACC	135	2433	GGUCCAGAGGCUUGUAGAG	398
2433	CGUGUGACGAGGAGCACGC	136	2433	CGUGUGACGAGGAGCACGC	136	2451	GCGUGCUCUCCUGUCACACG	399
2451	CUGGUCCUCCAUAGACUUGC	137	2451	CUGGUCCUCCAUAGACUUGC	137	2469	GCAAGUCAUUGGAGGACCAG	400
2469	CUGAAGCACACUCCUGCCA	138	2469	CUGAAGCACACUCCUGCCA	138	2487	UGGCAGGAGUGUGCUUCAG	401
2487	AGCCACCCUGACCCACCCUU	139	2487	AGCCACCCUGACCCACCCUU	139	2505	AGGGGUGGUCAGGGUGGCU	402
2505	UUGCUGCAGGACGCCCUCC	140	2505	UUGCUGCAGGACGCCCUCC	140	2523	GGAGGGCGUCCUGCAGCAA	403

2523	CGCAUCUCACAGAACUUC	141	2523	CGCAUCUCACAGAACUUC	141	2541	GGAAGUUCUGUGAGAGCG	404
2541	CUGUCCAGCAUCAAUGAGG	142	2541	CUGUCCAGCAUCAAUGAGG	142	2559	CCUCAUUGAUGCUGGACAG	405
2559	GAGAUACACACCCGACGGC	143	2559	GAGAUACACACCCGACGGC	143	2577	GCCGUGGGGUGUGAUCUC	408
2577	CAGUCCAGCAGGUGAAGA	144	2577	CAGUCCAGCAGGUGAAGA	144	2595	UCUACACCGUCAUGGACUG	407
2595	AAGGAGAGCAGCGGACG	145	2595	AAGGAGAGCAGCGGACG	145	2613	GCUGCGGUGCUCUCGCCU	408
2613	CUGCUGAAGGACAGCUCA	146	2613	CUGCUGAAGGACAGCUCA	146	2631	UGAAGCUGUCCUUCAGCAG	409
2631	AUGGUGAGCUGGUGAGG	147	2631	AUGGUGAGCUGGUGAGG	147	2649	CCUCCACAGCUCGCCACAU	410
2649	GGGGCCCGCAAGCUGCGCC	148	2649	GGGGCCCGCAAGCUGCGCC	148	2667	GGCGCAGCUCGGGGCCCC	411
2667	CACGUCUCCUGUUCACCG	149	2667	CACGUCUCCUGUUCACCG	149	2685	CGGUGAACAGGAGACGUG	412
2685	GAGCUGCUUCUCUGCACCA	150	2685	GAGCUGCUUCUCUGCACCA	150	2703	UGGUGCAGAGAAGCAGCUC	413
2703	AAGCUCAGAGAGCAGAGCG	151	2703	AAGCUCAGAGAGCAGAGCG	151	2721	CGCUCUGCUUCUUGAGCUU	414
2721	GGAGGCAAAACGCAGCAGU	152	2721	GGAGGCAAAACGCAGCAGU	152	2739	ACUGCUGCUUUUUGCCUCC	415
2739	UAUGACUGCAAAUGGUACA	153	2739	UAUGACUGCAAAUGGUACA	153	2757	UGUACCAUUGCAGUACA	416
2757	AUUCGCGUCAGGUAUCUA	154	2757	AUUCGCGUCAGGUAUCUA	154	2775	UGAGAUCGUGAGCGGAU	417
2775	AGCUUCCAGAGUGGUAUG	155	2775	AGCUUCCAGAGUGGUAUG	155	2793	CAUCCACCACUUGGAAGCU	418
2793	GAACUGGAGGCAGUGCCCA	156	2793	GAACUGGAGGCAGUGCCCA	156	2811	UGGGCACUGCCUCCAGUUC	419
2811	AACAUCCCCUGGUGCCCG	157	2811	AACAUCCCCUGGUGCCCG	157	2829	CGGGCACCAGGGGGAUGU	420
2829	GAUGAGGAGCUGGACGCUU	158	2829	GAUGAGGAGCUGGACGCUU	158	2847	AAGCGUCCAGCUCUACAUC	421
2847	UUGAAGAUCAAGAUCC	159	2847	UUGAAGAUCAAGAUCC	159	2865	GGGAGUUCUUGAUCUCAA	422
2865	CAGAUCAAGAGUACAUC	160	2865	CAGAUCAAGAGUACAUC	160	2883	GGAGUACACUUGAUCUG	423
2883	CAGAGAGAGAGAGGGCGA	161	2883	CAGAGAGAGAGAGGGCGA	161	2901	UCGCCUUCUUCUCUCUCUG	424
2901	AACAAGGCAGCAAGGCUA	162	2901	AACAAGGCAGCAAGGCUA	162	2919	UAGCCUUGCUGCCCUUGU	425
2919	ACGGAGAGGCUGAAGAAGA	163	2919	ACGGAGAGGCUGAAGAAGA	163	2937	UCUUCUUCAGCCUUCUCCGU	426
2937	AAGCUGUCGGAGCAGGAGU	164	2937	AAGCUGUCGGAGCAGGAGU	164	2955	ACUCCUGCUCGACAGCUU	427
2955	UCACUGCUGCUGCUUAUGU	165	2955	UCACUGCUGCUGCUUAUGU	165	2973	ACAUAAAGCAGCAGCAGUGA	428
2973	UCUCCAGCAUGGCCUJCA	166	2973	UCUCCAGCAUGGCCUJCA	166	2991	UGAAGGCCAUGCUGGGAGA	429
2991	AGGGUGCACAGCCGCAACG	167	2991	AGGGUGCACAGCCGCAACG	167	3009	CGUUGCGGCGUGGCAACCU	430
3009	GGCAAGAGUUAACAGUUC	168	3009	GGCAAGAGUUAACAGUUC	168	3027	GGAACGUGUAACUCUUGCC	431
3027	CUGAUCUCCUCUGACUUAUG	169	3027	CUGAUCUCCUCUGACUUAUG	169	3045	CAUAGUCAGAGGAGAUCA	432
3045	GAGCGUGCAGAGUGGAGGG	170	3045	GAGCGUGCAGAGUGGAGGG	170	3063	CCCUCCACUUCGACGCU	433
3063	GAGAACAUCCGGGAGCAGC	171	3063	GAGAACAUCCGGGAGCAGC	171	3081	GCUGCUCCGGGAUGUUCUC	434
3081	CAGAAGAAGUGUUAACAGAA	172	3081	CAGAAGAAGUGUUAACAGAA	172	3099	UUCUGAAACACUUCUUCUG	435
3099	AGCUUCUCCUCUGACAUCCG	173	3099	AGCUUCUCCUCUGACAUCCG	173	3117	CGGAUGUCAGGGAGAGCU	436
3117	GUGGAGCUGCAGAUCCUGA	174	3117	GUGGAGCUGCAGAUCCUGA	174	3135	UCAGCAUCUGCAGCUCCAC	437
3135	ACCAACUCUGUGUGAAAC	175	3135	ACCAACUCUGUGUGAAAC	175	3153	GUUUCACACAGGAGUUGU	438
3153	CUCCAGACUUGUCCACAGCA	176	3153	CUCCAGACUUGUCCACAGCA	176	3171	UGCUGGACACAGUCUGGAG	439

3171	AUUCGCGUGACCAUAUA	177	3171	AUUCGCGUGACCAUAUA	177	3189	UAUUGUGUGACGGGAU	440
3189	AGGAAGAUGAUGUCUC	178	3189	AAGGAAGAUGAUGUCUC	178	3207	GAGACUCAUCAUUCU	441
3207	CCGGGCGUCUAUGGUUUC	179	3207	CCGGGCGUCUAUGGUUUC	179	3225	GAAACCAUAGAGCCCGG	442
3225	CUGAAUGUCAUGUCCACU	180	3225	CUGAAUGUCAUGUCCACU	180	3243	AGUGGACGAUGACAUUCAG	443
3243	UCAGCCACUGGAUUAAGC	181	3243	UCAGCCACUGGAUUAAGC	181	3261	GCUIAAAUCCAGUGGCUGA	444
3261	CAGAGUUCAAUUCUGUACU	182	3261	CAGAGUUCAAUUCUGUACU	182	3279	AGUACAGAUUAGAACUCUG	445
3279	UGCACCCUGGAGGUGGAU	183	3279	UGCACCCUGGAGGUGGAU	183	3297	AUCCACCUCCAGGUGCA	446
3297	UCCUUUGGUUUAUUGUGA	184	3297	UCCUUUGGUUUAUUGUGA	184	3315	UCACAAAUACCCAAAGGA	447
3315	AUAAAGCAAAGACGCGG	185	3315	AUAAAGCAAAGACGCGG	185	3333	CGCGGUCUUUGCUUAU	448
3333	GUCUACAGGACACAGCUG	186	3333	GUCUACAGGACACAGCUG	186	3351	CAGCUGUGUCCUUGUAGAC	449
3351	GAGCCAAACUGGAACGAGG	187	3351	GAGCCAAACUGGAACGAGG	187	3369	CCUGUCCAGUUUGGCUC	450
3369	GAUUIUGAGUAGAGCUGG	188	3369	GAUUIUGAGUAGAGCUGG	188	3387	CCAGCUCUAUCUCAAUUC	451
3387	GAGGCUCCAGACCUGA	189	3387	GAGGCUCCAGACCUGA	189	3405	UCAGGGUCUGGAGCCUC	452
3405	AGGAUACUGUGCUAUGAAA	190	3405	AGGAUACUGUGCUAUGAAA	190	3423	UUIUCAUAGCACAGUAUCCU	453
3423	AAGUUAACAAGACGA	191	3423	AAGUUAACAAGACGA	191	3441	UCGUCUUGUUGUAACACU	454
3441	AAGUCCCAAGGAGGAGC	192	3441	AAGUCCCAAGGAGGAGC	192	3459	CGUCCUCCUUGGGGAUCUU	455
3459	GGCAGAGCACGGACAGAC	193	3459	GGCAGAGCACGGACAGAC	193	3477	GUCUUGCCUGUCUCUGCC	456
3477	CUCUUGGGAGGGCCAGG	194	3477	CUCUUGGGAGGGCCAGG	194	3495	CCUGGCCUUGCCCAUGAG	457
3495	GUCAGCUGGACCCGAGG	195	3495	GUCAGCUGGACCCGAGG	195	3513	CCUGCGGUCACGUCUGAC	458
3513	GCCUUGCAGGACAGACU	196	3513	GCCUUGCAGGACAGACU	196	3531	AGUCUCUGUCCUGCAGGGC	459
3531	UGGCAGCGCACCGUCAUCG	197	3531	UGGCAGCGCACCGUCAUCG	197	3549	CGAUGACGGUGCGCUGCCA	460
3549	GCCAUGAAUGGGAUCAAG	198	3549	GCCAUGAAUGGGAUCAAG	198	3567	CUUGAUCCCAUUCUAGGC	461
3567	GUAAAGCUCUGGUCAAGU	199	3567	GUAAAGCUCUGGUCAAGU	199	3585	ACUUGACCGAGAGCUUAC	462
3585	UUCAACAGCAGGGAGUUA	200	3585	UUCAACAGCAGGGAGUUA	200	3603	UGAACUCCUUGCUGUUGAA	463
3603	AGCUUGAAGAGGAGUCCGU	201	3603	AGCUUGAAGAGGAGUCCGU	201	3621	ACGGCAUCCUUCUUAAGCU	464
3621	UCCCGAAACAGACAGGGG	202	3621	UCCCGAAACAGACAGGGG	202	3639	CCCCUGUCUGUUUUCGGGA	465
3639	GUCUUCGGAGUCAAAGUUG	203	3639	GUCUUCGGAGUCAAAGUUG	203	3657	CAUUCUUUGACUCCGAAGC	466
3657	GCUGUGGUCACCAAGAGAG	204	3657	GCUGUGGUCACCAAGAGAG	204	3675	CUCUCUUUGGUGACCACAGC	467
3675	GAGAGGUCCAAGGUGCCCU	205	3675	GAGAGGUCCAAGGUGCCCU	205	3693	AGGGACCUUGGACCUCUC	468
3693	UACUUGGUGCGCCAGUGCG	206	3693	UACUUGGUGCGCCAGUGCG	206	3711	CGCACUGGCGCACCAUGUA	469
3711	GUGGAGGAGAUCCGCGCC	207	3711	GUGGAGGAGAUCCGCGCC	207	3729	GGCGCUCGAUCCUCCAC	470
3729	CGAGGCAUGGAGGAGGUGG	208	3729	CGAGGCAUGGAGGAGGUGG	208	3747	CCACCUCCUCCAUUGCCUCG	471
3747	GGCAUCUACCGGUGUCCG	209	3747	GGCAUCUACCGGUGUCCG	209	3765	CGGACACGGUAGAUCC	472
3765	GGUGUGGCCACGGACAUC	210	3765	GGUGUGGCCACGGACAUC	210	3783	GGAUGUCCGUGGCCACACC	473
3783	CAGGCACUGAAGGCAGCCU	211	3783	CAGGCACUGAAGGCAGCCU	211	3801	AGGCGCCUUCAGUGCCUCG	474
3801	UUCGACGUAUAUACAAGG	212	3801	UUCGACGUAUAUACAAGG	212	3819	CCUUGUUAUUGACGUGCAA	475

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4467	GGGCAGAUUCUGCCCGAGGU	249	4467	GGGCAGAUUCUGCCCGAGGU	249	4485	ACCUGGGCGAGAUUGCCG	512
4485	UCUGGGAGCCCGAGGCUUG	250	4485	UCUGGGAGCCCGAGGCUUG	250	4503	CCAGCCUGGGGCUCCAGAG	513
4503	GCCUCAGACUGUGGUUUU	251	4503	GCUCAGACUGUGGUUUU	251	4521	AAAAACCACAGUCUGAGGC	514
4521	UUAUGUGGCCACCCGAGGG	252	4521	UUAUGUGGCCACCCGAGGG	252	4539	CCUCGGGUGGCCACAUAA	515
4539	GCGCCCCAAGCCAGUUAU	253	4539	GCGCCCCAAGCCAGUUAU	253	4557	AUGAACUGGCUUGGGCGC	516
4557	UCUCAGAGUCCAGGCCUGA	254	4557	UCUCAGAGUCCAGGCCUGA	254	4575	UCAGCCUGGCUUCCAGAGA	517
4575	ACCCUGGGAGACAGGGUGA	255	4575	ACCCUGGGAGACAGGGUGA	255	4593	UCACCCUGUCCACAGGGU	518
4593	AAGGAGUGAUUUUAUGA	256	4593	AAGGAGUGAUUUUAUGA	256	4611	UCAUAAAAUACACUCCUU	519
4611	AACUUAACUUAAGAGUCUA	257	4611	AACUUAACUUAAGAGUCUA	257	4629	UUAAGACUCUAAAGUUAUU	520
4629	AAAGAUUUCUACUGGAUCA	258	4629	AAAGAUUUCUACUGGAUCA	258	4647	UGAUCCAGUAGAAUUCUUU	521
4647	ACUUGUCAAGAUCCGCCCU	259	4647	ACUUGUCAAGAUCCGCCCU	259	4665	AGGCGCAUCUUGACAAGU	522
4665	UCUCUGGGAGAGGGGAAC	260	4665	UCUCUGGGAGAGGGGAAC	260	4683	GUUCCUUCUCCCCAGAGA	523
4683	CGUGACCGGAUCCUCAC	261	4683	CGUGACCGGAUCCUCAC	261	4701	GUGAGGGAUCCGGUCACG	524
4701	CUGUUGUAUCUUGAAUAA	262	4701	CUGUUGUAUCUUGAAUAA	262	4719	UUUUAUCAAGAUACAACAG	525
4719	ACGCUUGCUUUAUCCUG	263	4719	ACGCUUGCUUUAUCCUG	263	4737	CAGGAUGAAGCAGAGCGGU	526

## NM\_005157 (ABL)

Pos	Target Sequence	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
3	CCUUCGCCCGCGAGGAUC	527	3	CCUUCGCCCGCGAGGAUC	527	21	GAUCCUCGCAGGGGAAGG	846
21	CGCCGUUGGCCCGGGUUG	528	21	CGCCGUUGGCCCGGGUUG	528	39	CCAACCCCGGGCCACGGCG	847
39	GCUUUGGAAAGCGCGGUG	529	39	GCUUUGGAAAGCGCGGUG	529	57	CACCGCCGCUUCCAAAGC	848
57	GGCUUUGGGCGGGCUUGG	530	57	GGCUUUGGGCGGGCUUGG	530	75	CCGAGCCCGGCCCAAGCC	849
75	GCCUCGGGAACGCCAGGGG	531	75	GCCUCGGGAACGCCAGGGG	531	93	CCCCUGGCUUCCCGAGGC	850
93	GCCCCUGGGUGCGGACGGG	532	93	GCCCCUGGGUGCGGACGGG	532	111	CCCGUCCGCACCCAGGGCG	851
111	GCGCGCCAGAGGGGUU	533	111	GCGCGCCAGAGGGGUU	533	129	AACCCCUCCUGGCGCGCG	852
129	UAAGGCAGGCGGCGCGC	534	129	UAAGGCAGGCGGCGCGC	534	147	CGCGCCGCGCUGCGCCUUA	853
147	GGGGCGGGCGGGCCUUG	535	147	GGGGCGGGCGGGCCUUG	535	165	CCAGGCCCGCCCGCCCGCC	854
165	GCGGGCGCCUUCGCGGC	536	165	GCGGGCGCCUUCGCGGC	536	183	GCCCGGAGGGCGCGCCCG	855
183	CCCUUUGUUAACAGCGCG	537	183	CCCUUUGUUAACAGCGCG	537	201	CGCGCCUGUUAACAAAGGG	856
201	GUCCCGCCAGCGGAGAGG	538	201	GUCCCGCCAGCGGAGAGG	538	219	CGUCUCCGUGGCCGGGAC	857
219	GCGGCCGCCUGGGCGGGC	539	219	GCGGCCGCCUGGGCGGGC	539	237	GCCCGCCAGGGCGGGCGC	858
237	GCGGGCGGGCGGGCGCG	540	237	GCGGGCGGGCGGGCGCG	540	255	CCGCGCCCGCGCGCGCGC	859
255	GUGAGGCGGCGGCGGGG	541	255	GUGAGGCGGCGGCGGGG	541	273	CCCGCAGGCGCGCGCCAC	860
273	GCGGCGCGCGGGCGGGG	542	273	GCGGCGCGCGGGCGGGG	542	291	CCCGGCCCCCGGGCGCGC	861

291	GCCGAGCCGGCCUAGCC	543	291	GCCGAGCCGGCCUAGCC	543	309	GGCUCAGGCCCGGCUCCGGC	862
308	CGGGCCCGACCGAGCUGG	544	309	CGGGCCCGACCGAGCUGG	544	327	CCAGCUCGGUCCGGGCCCCG	863
327	GGAGAGGGCUCGCGCCG	545	327	GGAGAGGGCUCGCGCCG	545	345	CGGGCCGAGCCCCUCUCC	864
345	GAUGUUCGCUUGGCGAA	546	345	GAUGUUCGCUUGGCGAA	546	363	UUGGCCAAGCGAACGAUC	865
363	AAUGUUGGAGAGUCCCU	547	363	AAUGUUGGAGAGUCCCU	547	381	AGGAGAUCCCAACAUUU	866
381	UGAAGCUUGGGCUGCAA	548	381	UGAAGCUUGGGCUGCAA	548	399	UUGAGCCCCACAGCUCA	867
399	AAUCCAAGAAGGGCUGUC	549	399	AAUCCAAGAAGGGCUGUC	549	417	GACAGCCCCUUCUUGGAUU	868
417	CCUGUCCUCCAGCUGUA	550	417	CCUGUCCUCCAGCUGUA	550	435	UAACAGCCUGGAGGACGAGG	869
435	AUCUGGAAGAAGCCUUA	551	435	AUCUGGAAGAAGCCUUA	551	453	UGAAGGGCUUCUCCAGAU	870
453	AGCGCCAGUAGCAUCUGA	552	453	AGCGCCAGUAGCAUCUGA	552	471	UCAGAUGCUACUGGCCGCU	871
471	ACUUUGAGCCUCAGGGUCU	553	471	ACUUUGAGCCUCAGGGUCU	553	489	AGACCCUGAGGCUCAAAAGU	872
489	UGAGUGAAGCCGUCGUG	554	489	UGAGUGAAGCCGUCGUG	554	507	CAACGAGCGCUUCACUCA	873
507	GGAACUCCAAGGAAACCU	555	507	GGAACUCCAAGGAAACCU	555	525	AGGUUUUCCUUGGAGUCC	874
525	UUCUCGCGGACCCAGUGA	556	525	UUCUCGCGGACCCAGUGA	556	543	UCACUGGGUCCAGCGAGAA	875
543	AAAUGACCCCAACCUUUU	557	543	AAAUGACCCCAACCUUUU	557	561	AAAAGGUUGGGGUCAUUU	876
561	UCGUUGCACUGUAUGAUUU	558	561	UCGUUGCACUGUAUGAUUU	558	579	AAAUCAUACAGUGCAACGA	877
579	UUGUGGCCAGUGGAGUAA	559	579	UUGUGGCCAGUGGAGUAA	559	597	UUAUCUCCACUGGCCACAA	878
597	ACACUCUAAGCAUAACUAA	560	597	ACACUCUAAGCAUAACUAA	560	615	UUAGUUUAGCUUAGAGUGU	879
615	AAGGUGAAAAGCUCGCGGU	561	615	AAGGUGAAAAGCUCGCGGU	561	633	ACCCGAGCUCUUCACCCUU	880
633	UCUAGGCUAUAAUCACAA	562	633	UCUAGGCUAUAAUCACAA	562	651	UUGUGAUUUAAGCCUAAAG	881
651	AUGGGGAUUGGUGAAGC	563	651	AUGGGGAUUGGUGAAGC	563	669	GCUUCACACCAUUCGCCAU	882
669	CCCAACCAAAAUGGCCA	564	669	CCCAACCAAAAUGGCCA	564	687	UGGCCAUUUUUGGUUUUGG	883
687	AAGGCUUGGUCGCAAGCAA	565	687	AAGGCUUGGUCGCAAGCAA	565	705	UUGCUUGGGACCCAGCCUU	884
705	ACUACAUCACGCCAGUCA	566	705	ACUACAUCACGCCAGUCA	566	723	UUGACUGGCGUGAUGUAGU	885
723	ACAGUCUGGAGAAACACUC	567	723	ACAGUCUGGAGAAACACUC	567	741	GAGUGUUUUCUCCAGACUGU	886
741	CCUGGUACCAUGGGCCUGU	568	741	CCUGGUACCAUGGGCCUGU	568	759	ACAGGCCCAUGGUACCCAGG	887
759	UGUCCCGCAUUGCCGCUA	569	759	UGUCCCGCAUUGCCGCUA	569	777	UCAGCGCAUUGCGGGACA	888
777	AGUAUCCGCUAGCAGCGG	570	777	AGUAUCCGCUAGCAGCGG	570	795	CCGCUGCUCAGCGGAUACU	889
795	GGAUCAUUGGCAGCUUCUU	571	795	GGAUCAUUGGCAGCUUCUU	571	813	AAGAAGCUCGCAUUGAUCC	890
813	UGGUGCGUGAGAGUGAGAG	572	813	UGGUGCGUGAGAGUGAGAG	572	831	CUCUACUCUCACGCGACCA	891
831	GCAGUCCUAGCCAGAGGUC	573	831	GCAGUCCUAGCCAGAGGUC	573	849	GACCUCUGGCUAGGACUCC	892
849	CCAUCUCGCUAGAGUACGA	574	849	CCAUCUCGCUAGAGUACGA	574	867	UCGUUUCUACGCGAGAUUG	893
867	AAGGAGGGUGUACCAUUA	575	867	AAGGAGGGUGUACCAUUA	575	885	UAAUGGUACACCCUCCUU	894
885	ACAGGAUCAACACUGCUUC	576	885	ACAGGAUCAACACUGCUUC	576	903	GAAGCAGUGUUGAUCCUGU	895
903	CUGAUGGCAAGCUCUACGU	577	903	CUGAUGGCAAGCUCUACGU	577	921	ACGUAGAGCUCUGCCAUACG	896
921	UCUCCUCCGAGAGCCGCUU	578	921	UCUCCUCCGAGAGCCGCUU	578	939	AAGCGGCUCUCGGAGGAGA	897

939	UAAACACCCUGGCCGAGUU	579	939	UAAACACCCUGGCCGAGUU	579	957	898
957	UGGUUUAUUAUUAUUAAC	580	967	UGGUUUAUUAUUAUUAAC	580	975	899
975	CGGUGGCCGACGGGCUCAU	581	975	CGGUGGCCGACGGGCUCAU	581	993	900
993	UACACACGCUCCAUUAUCC	582	993	UACACACGCUCCAUUAUCC	582	1011	901
1011	CAGCCCAAAAGCGCAACAA	583	1011	CAGCCCAAAAGCGCAACAA	583	1029	902
1029	AGCCACUGUCUAUGGUGU	584	1029	AGCCACUGUCUAUGGUGU	584	1047	903
1047	UGUCCCCAACUACGACAA	585	1047	UGUCCCCAACUACGACAA	585	1065	904
1065	AGUGGAGAUUGAACGCAC	586	1065	AGUGGAGAUUGAACGCAC	586	1083	905
1083	CGGACAUACCAUGAAGCA	587	1083	CGGACAUACCAUGAAGCA	587	1101	906
1101	ACAAGCUGGGCGGGGCCA	588	1101	ACAAGCUGGGCGGGGCCA	588	1119	907
1119	AGUACGGGAGGUGUACGA	589	1119	AGUACGGGAGGUGUACGA	589	1137	908
1137	AGGGCGUGUGGAAGAAUA	590	1137	AGGGCGUGUGGAAGAAUA	590	1155	909
1155	ACAGCCUGACGGUGGCCGU	591	1155	ACAGCCUGACGGUGGCCGU	591	1173	910
1173	UGAAGACCUUGAAGGAGGA	592	1173	UGAAGACCUUGAAGGAGGA	592	1191	911
1191	ACACCAUGGAGGUGGAAGA	593	1191	ACACCAUGGAGGUGGAAGA	593	1209	912
1209	AGUUCUUGAAAGAACGUGC	594	1209	AGUUCUUGAAAGAACGUGC	594	1227	913
1227	CAGUCAUGAAAGAGAUCAA	595	1227	CAGUCAUGAAAGAGAUCAA	595	1245	914
1245	AACACCCUAAACUAGUGCA	596	1245	AACACCCUAAACUAGUGCA	596	1263	915
1263	AGCUCCUUGGGUGUCGCAC	597	1263	AGCUCCUUGGGUGUCGCAC	597	1281	916
1281	CCGGGAGCCCCGUUCUA	598	1281	CCGGGAGCCCCGUUCUA	598	1299	917
1299	AUAUCAUCACUGAGUUAU	599	1299	AUAUCAUCACUGAGUUAU	599	1317	918
1317	UGACCUACGGGAACCUCCU	600	1317	UGACCUACGGGAACCUCCU	600	1335	919
1335	UGGACUACCUAGGGAGUG	601	1335	UGGACUACCUAGGGAGUG	601	1353	920
1353	GCAACCGGCAGGAGGUGAA	602	1353	GCAACCGGCAGGAGGUGAA	602	1371	921
1371	ACGCCGUGGUGUCUGUA	603	1371	ACGCCGUGGUGUCUGUA	603	1389	922
1389	ACAUGGCCACUCAGAUUC	604	1389	ACAUGGCCACUCAGAUUC	604	1407	923
1407	CGUACGCCAUGGAGUACCU	605	1407	CGUACGCCAUGGAGUACCU	605	1425	924
1425	UAGAGAAGAAAAACUUAU	606	1425	UAGAGAAGAAAAACUUAU	606	1443	925
1443	UCCACAGAGAUUCUUGCUG	607	1443	UCCACAGAGAUUCUUGCUG	607	1461	926
1461	CCGAAACUGCCUGGUAGG	608	1461	CCGAAACUGCCUGGUAGG	608	1479	927
1479	GGGAGAACCAUUGGUGAA	609	1479	GGGAGAACCAUUGGUGAA	609	1497	928
1497	AGGUAGCUGAUUUUGGCCU	610	1497	AGGUAGCUGAUUUUGGCCU	610	1515	929
1515	UGAGCAGGUUGAUGACAGG	611	1515	UGAGCAGGUUGAUGACAGG	611	1533	930
1533	GGGACACCUACACAGCCCA	612	1533	GGGACACCUACACAGCCCA	612	1551	931
1551	AUGCUGGAGCCAAUUCUCC	613	1551	AUGCUGGAGCCAAUUCUCC	613	1569	932
1569	CCAUCAAUUGGACUGCACC	614	1569	CCAUCAAUUGGACUGCACC	614	1587	933

1587	CCGAGAGCCUGGGCCUACAA	615	1587	CCGAGAGCCUGGGCCUACAA	615	1605	UUGUAGCCAGGCUCUCGG	934
1605	ACAAGUUCUCCAUCAAGUC	616	1605	ACAAGUUCUCCAUCAAGUC	616	1623	GACUUGAUGGAGAACUUGU	935
1623	CCGACGUCUGGGCAUUUGG	617	1623	CCGACGUCUGGGCAUUUGG	617	1641	CCAAAUGCCAGACGUCGG	936
1641	GAGUUAUGCUUUGGAAAU	618	1641	GAGUUAUGCUUUGGAAAU	618	1659	AUUUCCCAAAGCAUACUC	937
1659	UUGCUACCUAUGGCAUGUC	619	1659	UUGCUACCUAUGGCAUGUC	619	1677	GACAUCCAUAGGUAGCAA	938
1677	CCCUUACCCGGGAUUGA	620	1677	CCCUUACCCGGGAUUGA	620	1695	UCAAUCCCGGGUAAGGG	939
1695	ACCGUCCCGAGGUGAUGA	621	1695	ACCGUCCCGAGGUGAUGA	621	1713	UCAUACCCUGGGAAACGGU	940
1713	AGCUGCUAGAGAAGGACUA	622	1713	AGCUGCUAGAGAAGGACUA	622	1731	UAGUCCUUCUCUAGCAGCU	941
1731	ACCGCAUGAAGCGCCAGA	623	1731	ACCGCAUGAAGCGCCAGA	623	1749	UCUGGCGCUUUAUGCGGU	942
1749	AAGCGUCCCGAGAGAGGU	624	1749	AAGCGUCCCGAGAGAGGU	624	1767	ACCUUCUCUGGGCAGCCUU	943
1767	UCUAUGAACUCAUGCGAGC	625	1767	UCUAUGAACUCAUGCGAGC	625	1785	GCUCGCAUGAGUUAUAGA	944
1785	CAUGUUGGCAGUGGAUCC	626	1785	CAUGUUGGCAGUGGAUCC	626	1803	GGAUCCACUGCCAACAU	945
1803	CCUCUGACCGGCCUCCUU	627	1803	CCUCUGACCGGCCUCCUU	627	1821	AAGGAGGCGCGUCAGAGG	946
1821	UUGCUGAAAUCCACCAAGC	628	1821	UUGCUGAAAUCCACCAAGC	628	1839	GCUUGGUGGAUUAUCAGCAA	947
1839	CCUUUGAAACAAUUGUCCA	629	1839	CCUUUGAAACAAUUGUCCA	629	1857	UGGAACAUUUGUUAUCAAAGG	948
1857	AGGAUCCAGUAUCUCAGA	630	1857	AGGAUCCAGUAUCUCAGA	630	1875	UCUGAGAUACUGGAUUCUU	949
1875	ACGAAGUGGAAAGGAGCU	631	1875	ACGAAGUGGAAAGGAGCU	631	1893	AGUCCUUUUCACCUUCGU	950
1893	UGGGAAACAAAGGCGUCCG	632	1893	UGGGAAACAAAGGCGUCCG	632	1911	CGGACGCUUUGUUUCCCA	951
1911	GUGGGCUGUGACUACCUU	633	1911	GUGGGCUGUGACUACCUU	633	1929	AAGUAGUCACAGCCCCAC	952
1929	UGCUGAGGCGCCAGAGCU	634	1929	UGCUGAGGCGCCAGAGCU	634	1947	AGCUCUGGGGCCUUGCAGCA	953
1947	UGCCACCAAGACGAGGAC	635	1947	UGCCACCAAGACGAGGAC	635	1965	GUCCUCGUCUUGGUGGGCA	954
1965	CCUCCAGGAGAGCUGCAGA	636	1965	CCUCCAGGAGAGCUGCAGA	636	1983	UCUGCAGCUCUCCUGGAGG	955
1983	AGCACAGAGACACCACUGA	637	1983	AGCACAGAGACACCACUGA	637	2001	UCAGUGGUGUCUCUGUGCU	956
2001	ACGUGCCUGAGAUGCCUCA	638	2001	ACGUGCCUGAGAUGCCUCA	638	2019	UGAGGCAUCUCAGGCACGU	957
2019	ACUCCAAAGGCCAGGGAGA	639	2019	ACUCCAAAGGCCAGGGAGA	639	2037	UCUCCUUGGCCCUUGGAGU	958
2037	AGAGCGAUCCUUGGACCA	640	2037	AGAGCGAUCCUUGGACCA	640	2055	UGGUCACAGAGGAUCGUCU	959
2055	AUGAGCCUCCGUGUCUCC	641	2055	AUGAGCCUCCGUGUCUCC	641	2073	GGAGACACGGCAGGCUCAU	960
2073	CAUUGCUCCUUGGAAAGA	642	2073	CAUUGCUCCUUGGAAAGA	642	2091	UCUUUUGAGGGAGGCAU	961
2091	AGCGAGGUCUCCCGGAGGG	643	2091	AGCGAGGUCUCCCGGAGGG	643	2109	CCCUCCGGGGGACCUCCGU	962
2109	GCGGCCUGAAUAGAUGA	644	2109	GCGGCCUGAAUAGAUGA	644	2127	UCAUCUUAUUAAGGCCGC	963
2127	AGCGCCUUCUCCCAAGA	645	2127	AGCGCCUUCUCCCAAGA	645	2145	UCUUUGGGGAGAGGCGCU	964
2145	ACAAAAGACCAACUUGUU	646	2145	ACAAAAGACCAACUUGUU	646	2163	AACAAGUUGGUCUUUUUGU	965
2163	UCAGCGCCUUGAUAAGAA	647	2163	UCAGCGCCUUGAUAAGAA	647	2181	UUCUUGAUAAGGCGCUGA	966
2181	AGAAGAAGAAGACAGCCCC	648	2181	AGAAGAAGAAGACAGCCCC	648	2199	GGGCGUCUUCUUCUUCU	967
2199	CAACCCUCCCAACAGCAG	649	2199	CAACCCUCCCAACAGCAG	649	2217	CUGCGUUGGGAGGGGUUG	968
2217	GCAGCUCCUUCGGGAGAU	650	2217	GCAGCUCCUUCGGGAGAU	650	2235	AUCUCCCGGAAGGAGCUGC	969

2235	UGGACGGCCAGCCGGAGCG	651	2235	UGGACGGCCAGCCGGAGCG	651	2253	CGCUCGGGCGUGGGCCGUCCA	970
2253	GCAGAGGGCCGGCGAGGA	652	2253	GCAGAGGGCCGGCGAGGA	652	2271	UCCUCGGCCGGCCCUUGC	971
2271	AAGAGGGCCGAGACUACG	653	2271	AAGAGGGCCGAGACUACG	653	2289	CUGAUGUCUGGGCCCUU	972
2289	GCAACGGGGCACUGGCUU	654	2289	GCAACGGGGCACUGGCUU	654	2307	AAAGCCAGUGCCCGUUGC	973
2307	UACCCCCUUGGACACAGC	655	2307	UACCCCCUUGGACACAGC	655	2325	GCUGUCCAAAGGGGGUGA	974
2325	CUGACCCAGCCAAUCCCC	656	2325	CUGACCCAGCCAAUCCCC	656	2343	GGGAGUCCGUGGGGUCAG	975
2343	CAAAGCCAGCAUUGGGC	657	2343	CAAAGCCAGCAUUGGGC	657	2361	GCCCAUUGGUGGCUUUG	976
2361	CUGGGUCCCCAAUUGAGC	658	2361	CUGGGUCCCCAAUUGAGC	658	2379	GCUCCAUUGGGGACCCAG	977
2379	CCCUCGGGAGUCCGGGG	659	2379	CCCUCGGGAGUCCGGGG	659	2397	CCCCGGACUCCCGAGGG	978
2397	GCUCAGGCUCCGGUCC	660	2397	GCUCAGGCUCCGGUCC	660	2415	GGAGACGGAAGCCUAGC	979
2415	CCCACCUUGGAAAGUUC	661	2415	CCCACCUUGGAAAGUUC	661	2433	GACUUCUCCACAGGUGG	980
2433	CCAGCACGUGACACAGC	662	2433	CCAGCACGUGACACAGC	662	2451	CUGCUGGUCAGCGUGCUG	981
2451	GCCGCCUAGCCACCGCGA	663	2451	GCCGCCUAGCCACCGCGA	663	2469	UCGCCGUGGCUAGGCGGC	982
2469	AGGAGGAGGGCGUGGCAG	664	2469	AGGAGGAGGGCGUGGCAG	664	2487	CUGCCACCGCCCUCCUCCU	983
2487	GCUCAGCAAGCGCUCCU	665	2487	GCUCAGCAAGCGCUCCU	665	2505	AGGAAGCGCUUGCUGGAGC	984
2505	UGCGCUUGCUCGUCUC	666	2505	UGCGCUUGCUCGUCUC	666	2523	GAGACGAGCAAGAGCGCA	985
2523	CCUGCGUCCCAUUGGGC	667	2523	CCUGCGUCCCAUUGGGC	667	2541	GCCCCAUGGGGAACGCGAG	986
2541	CCAAGGACACGGAGUGGAG	668	2541	CCAAGGACACGGAGUGGAG	668	2559	CUCCACUCCGUGCUUUG	987
2559	GGUCAGUCACGUGCCUCG	669	2559	GGUCAGUCACGUGCCUCG	669	2577	CGAGGACGUGACUGACC	988
2577	GGGACUUGCAGUCCAGGG	670	2577	GGGACUUGCAGUCCAGGG	670	2595	CCCGUGGACUGCAAGUCCC	989
2595	GAAGACAGUUGACUCGUC	671	2595	GAAGACAGUUGACUCGUC	671	2613	GACGAGUCAACUGUCUUC	990
2613	CCACAUUUGGAGGACAA	672	2613	CCACAUUUGGAGGACAA	672	2631	UUGUCCCUCCAAUUGUG	991
2631	AAAGUGAGAGCCGGCUCU	673	2631	AAAGUGAGAGCCGGCUCU	673	2649	AGAGCCGCUUCUCACUJU	992
2649	UGCCUCGGAAGGGGACGG	674	2649	UGCCUCGGAAGGGGACGG	674	2667	CCUGCCCUUCUCCGAGGCA	993
2667	GGGAGAACAGGUCUGACCA	675	2667	GGGAGAACAGGUCUGACCA	675	2685	UGGUCAGACCUUGUUCUCC	994
2685	AGGUGACCCGAGGCACAGU	676	2685	AGGUGACCCGAGGCACAGU	676	2703	ACUGUGCCUCCGGGUCACCU	995
2703	UAACGCCUCCCCCAGGCU	677	2703	UAACGCCUCCCCCAGGCU	677	2721	AGCCUGGGGGAGGCGUUA	996
2721	UGGUGAAAAAGAAUGAGGA	678	2721	UGGUGAAAAAGAAUGAGGA	678	2739	UCCUCAUUCUUUUCACCA	997
2739	AAGCUGCUGAUGAGGUCU	679	2739	AAGCUGCUGAUGAGGUCU	679	2757	AAGACCUCAUCAGCAGCUU	998
2757	UCAAGACAUCAUGGAGUC	680	2757	UCAAGACAUCAUGGAGUC	680	2775	GACUCCAUGAUGUJUUGA	999
2775	CCAGCCGGGCUCCAGCCC	681	2775	CCAGCCGGGCUCCAGCCC	681	2793	GGGCUAGGAGCCCGGGCUGG	1000
2793	CGCCCAACCUAGACUCCAA	682	2793	CGCCCAACCUAGACUCCAA	682	2811	UUUGGAGUCAGGUUGGGCG	1001
2811	AACCCUCCGGCGGAGGU	683	2811	AACCCUCCGGCGGAGGU	683	2829	ACCUGCCCGGAGGGGUU	1002
2829	UCACCGUGGCCCUUGCCUC	684	2829	UCACCGUGGCCCUUGCCUC	684	2847	GAGGACGGGGCCACGGUGA	1003
2847	CGGGCCUCCCCACAAAGGA	685	2847	CGGGCCUCCCCACAAAGGA	685	2865	UCCUUGUGGGGGAGGCGCG	1004
2865	AAGAAGCCUUGAAAGGCAG	686	2865	AAGAAGCCUUGAAAGGCAG	686	2883	CUGCCUUUCCAGGCUUCU	1005

2883	GUGCCUUAAGGGACCCUUCG	687	2883	GUGCCUUAAGGGACCCUUCG	687	2901	GCAGGGUCCCUAAGGCAC	1006
2901	CUGCAGCUGAGCCAGUGAC	688	2901	CUGCAGCUGAGCCAGUGAC	688	2919	GUCACUGGCUCAGCUGCAG	1007
2919	CCCCACCAAGCAAGCAGG	689	2919	CCCCACCAAGCAAGCAGG	689	2937	CCUGCUUUGCUGGUGGGG	1008
2937	GCUCAGGUGCAACCAAGGGG	690	2937	GCUCAGGUGCAACCAAGGGG	690	2955	CCCCUUGGUGCACCUGAGC	1009
2955	GCACCAAGGAGGCCCCCGC	691	2955	GCACCAAGGAGGCCCCCGC	691	2973	GCGGGCCCCUUGCUGGUGC	1010
2973	CCGAGGAGUCCAGAGUGAG	692	2973	CCGAGGAGUCCAGAGUGAG	692	2991	CUCACUGGAGACUCCUCCG	1011
2991	GGAGGCACAAGCACUCCUC	693	2991	GGAGGCACAAGCACUCCUC	693	3009	GAGGAGUGCUUGUGCCUCC	1012
3009	CUGAGUCGCCAGGGAGGGA	694	3009	CUGAGUCGCCAGGGAGGGA	694	3027	UCCUCCUUGGCGACUCAG	1013
3027	ACAAGGGGAAUUGUCCAA	695	3027	ACAAGGGGAAUUGUCCAA	695	3045	UUGGACAAUUCUCCUUGU	1014
3045	AGCUCAAACUUGCCCCGCC	696	3045	AGCUCAAACUUGCCCCGCC	696	3063	GGCGGGCAGGUUUGAGCU	1015
3063	CGCCCCACCAAGCAGCCUC	697	3063	CGCCCCACCAAGCAGCCUC	697	3081	GAGGCUUGGUGGGGGCG	1016
3081	CUGCAGGGAAGGCUUGAGG	698	3081	CUGCAGGGAAGGCUUGAGG	698	3099	CCUCCAGCCUUCUCCUCCAG	1017
3099	GAAAGCCUUCGACAGGCC	699	3099	GAAAGCCUUCGACAGGCC	699	3117	GGCCUCUGCGAGGGCUUUC	1018
3117	CGGCCAGGAGGCUUGCCGG	700	3117	CGGCCAGGAGGCUUGCCGG	700	3135	CGGCAGCCUCCUGGCCGG	1019
3135	GGGAGGAGUUGGGCGC	701	3135	GGGAGGAGUUGGGCGC	701	3153	GGCCCCAAGACUCCUCC	1020
3153	CAAGACAAAGCCACGAG	702	3153	CAAGACAAAGCCACGAG	702	3171	CUCGUGGCUUUGUCUUG	1021
3171	GUCUGGUUGAUGCUGGAA	703	3171	GUCUGGUUGAUGCUGGAA	703	3189	UUCACAGCAUCAACACAG	1022
3189	ACAGUGACGUGCCAAAGCC	704	3189	ACAGUGACGUGCCAAAGCC	704	3207	GGCUUGGACGUCACUUG	1023
3207	CCAGCCAGCGGACAGGGG	705	3207	CCAGCCAGCGGACAGGGG	705	3225	CCCUCUGCCGCGUGGUGG	1024
3225	GCCUAAAAGCCGUGCU	706	3225	GCCUAAAAGCCGUGCU	706	3243	AGCACGGGCUUUGAGGC	1025
3243	UCCCGGCCACUCCAAAGCC	707	3243	UCCCGGCCACUCCAAAGCC	707	3261	GGCUUUGGAGUGGCCGGGA	1026
3261	CACACCCCGCAAGCCGUC	708	3261	CACACCCCGCAAGCCGUC	708	3279	GACGGCUUGCGGGGUGUG	1027
3279	CGGGACCCCAUACAGCCC	709	3279	CGGGACCCCAUACAGCCC	709	3297	GGGCUAGUGGGGUGCCCG	1028
3297	CAGCCCCGUGCCCUUUC	710	3297	CAGCCCCGUGCCCUUUC	710	3315	GAAAGGGAACGGGGGUG	1029
3315	CCAGGUUGCCAUACAGCUC	711	3315	CCAGGUUGCCAUACAGCUC	711	3333	GAUGCUGAUGGCAACGUGG	1030
3333	CCUGGGCUUGGACAGGGGA	712	3333	CCUGGGCUUGGACAGGGGA	712	3351	UCCCUUGCCAAAGGCCAGG	1031
3351	ACCAGCCGUCUCCACUGC	713	3351	ACCAGCCGUCUCCACUGC	713	3369	GCAGUGGAAGACGGCUGGU	1032
3369	CCUUCAUCCUCCAUUAUC	714	3369	CCUUCAUCCUCCAUUAUC	714	3387	GAUAGAGAGGGGAUGAAGG	1033
3387	CAACCCGAGUGUCUUCG	715	3387	CAACCCGAGUGUCUUCG	715	3405	CGAAGAGACACUCCGUGU	1034
3405	GGAAACCCGACGCCUCC	716	3405	GGAAACCCGACGCCUCC	716	3423	GGAGGCUUGCGGGUUUUC	1035
3423	CAGAGCGGGCCAGGGCGC	717	3423	CAGAGCGGGCCAGGGCGC	717	3441	GCGCCUGGGCCCCGUCUG	1036
3441	CCAUCACCAAGGGCGUGGU	718	3441	CCAUCACCAAGGGCGUGGU	718	3459	ACCACGCCCUUGGUGAUGG	1037
3459	UCUUGGACAGCACCGAGGC	719	3459	UCUUGGACAGCACCGAGGC	719	3477	GCCUCGGUGCUGUCCAAAG	1038
3477	CGCUGGCCUCGCCAUUC	720	3477	CGCUGGCCUCGCCAUUC	720	3495	GAGUUGCGAGGCACAGCG	1039
3495	CUGGGAACUCCGAGCAGAU	721	3495	CUGGGAACUCCGAGCAGAU	721	3513	AUCUGCUCGGAGUCCAG	1040
3513	UGGCCAGCCACAGCGCAGU	722	3513	UGGCCAGCCACAGCGCAGU	722	3531	ACUGGCGUGGCGUGGCCA	1041

3531	UGCUGGAGCGCGGCAAAA	723	3531	UGCUGGAGCGCGGCAAAA	723	3549	UUUUUGCCGGCCUCCAGCA	1042
3549	ACGCUACACGCUUCUGCGU	724	3549	ACGCUACACGCUUCUGCGU	724	3567	ACGAGAACGUGUAGAGGU	1043
3567	UGAGCUAUGUGGAUCCAU	725	3567	UGAGCUAUGUGGAUCCAU	725	3585	AUGGAUCCACAUAGCUCA	1044
3585	UCCAGCAAAUGAGGAACAA	726	3585	UCCAGCAAAUGAGGAACAA	726	3603	UUUUUCCUCAUUUGCUGGA	1045
3603	AGUUUGCCUUCGAGAGGC	727	3603	AGUUUGCCUUCGAGAGGC	727	3621	GCCUCCGGAAGGCAACU	1046
3621	CCAUCAACAACUUGGAGAA	728	3621	CCAUCAACAACUUGGAGAA	728	3639	UUCUCCAGCUUUGUUGAUGG	1047
3639	AUAUUCUCCGGGAGCUCA	729	3639	AUAUUCUCCGGGAGCUCA	729	3657	UGAAGCUCUCCGGGAGAUU	1048
3657	AGAUCUGCCCGGCGUCAGC	730	3657	AGAUCUGCCCGGCGUCAGC	730	3675	GCUGACGCCGGGCGAGUUC	1049
3675	CAGGCAGUGGUCGCGGCG	731	3675	CAGGCAGUGGUCGCGGCG	731	3693	GCCGCGGACCCACUGCCUG	1050
3693	CCACUCAGGACUUCAGCAA	732	3693	CCACUCAGGACUUCAGCAA	732	3711	UUGCUGAAGUCCUGAGUGG	1051
3711	AGCUCUCAGUUCGGUGAA	733	3711	AGCUCUCAGUUCGGUGAA	733	3729	UUCACCGAACUGAGGAGCU	1052
3729	AGGAAUUCAGUACAUAGU	734	3729	AGGAAUUCAGUACAUAGU	734	3747	ACUAUGACACUGAUUUCU	1053
3747	UGCAGAGGAGCAGCAGUC	735	3747	UGCAGAGGAGCAGCAGUC	735	3765	GACUGCUGCUACCCUUGCA	1054
3765	CAGGGUCAGGUGUCAGGC	736	3765	CAGGGUCAGGUGUCAGGC	736	3783	GCCUGACACUAGACCCUG	1055
3783	CCGUCGGAGCUGCCUGCA	737	3783	CCGUCGGAGCUGCCUGCA	737	3801	UGCAGGCAGCUCGACGCGG	1056
3801	AGCACAUGCGGCGUCGCCC	738	3801	AGCACAUGCGGCGUCGCCC	738	3819	GGCGAGCCCGCAUGUGCU	1057
3819	CAUACCAUGACAGUGGCU	739	3819	CAUACCAUGACAGUGGCU	739	3837	AGCCACUGCAUGGGUUG	1058
3837	UGAGAAGGAGUAGUGAGU	740	3837	UGAGAAGGAGUAGUGAGU	740	3855	ACUCACUAGUCCUUCUCA	1059
3855	UCAGCACCUUGGCCCAGGA	741	3855	UCAGCACCUUGGCCCAGGA	741	3873	UCCUGGGCCAAAGGUGCUGA	1060
3873	AGCUCUGCGCCAGGCAGAG	742	3873	AGCUCUGCGCCAGGCAGAG	742	3891	CUCUGCCUGGCGCAGAGCU	1061
3891	GCUGAGGGCCCUUGGAGU	743	3891	GCUGAGGGCCCUUGGAGU	743	3909	ACUCCACAGGGCCCUACAGC	1062
3909	UCCAGCUCUACUACCUACG	744	3909	UCCAGCUCUACUACCUACG	744	3927	CGUAGGUAGUAGAGCUGGA	1063
3927	GUUUGCACCGCCUGCCUUC	745	3927	GUUUGCACCGCCUGCCUUC	745	3945	GAGGGCAGGGCGGUGCAAC	1064
3945	CCGCGACCUUCCUCCUCC	746	3945	CCGCGACCUUCCUCCUCC	746	3963	GGGAGGAGGAAGGUGCGGG	1065
3963	CCGCUCCGUCUCUGUCCUC	747	3963	CCGCUCCGUCUCUGUCCUC	747	3981	GAGGACAGAGACGCGGCGG	1066
3981	CGAAUUUUUAUCUGUGGAGU	748	3981	CGAAUUUUUAUCUGUGGAGU	748	3999	ACUCCACAGAUAAAUAUCG	1067
3999	UUCUUGCUCGUGGACUUC	749	3999	UUCUUGCUCGUGGACUUC	749	4017	GCAGUCCACGGAGCAGGAA	1068
4017	CAGUCGGCAUGCCAGGACC	750	4017	CAGUCGGCAUGCCAGGACC	750	4035	GGUCCUGGCAUGCCGACUG	1069
4035	CCGCCAGCCCGCUCGCCAC	751	4035	CCGCCAGCCCGCUCGCCAC	751	4053	GUGGGAGCGGGGCGUGCGG	1070
4053	CCUAGUGCCCGCAGACUAG	752	4053	CCUAGUGCCCGCAGACUAG	752	4071	CUCAGUCUGGGGCGACUAG	1071
4071	GCUCUCCAGGCCAGGUGGG	753	4071	GCUCUCCAGGCCAGGUGGG	753	4089	CCCACCUUGGCCUGGAGAGC	1072
4089	GAACGGCUGAUGUGGACUG	754	4089	GAACGGCUGAUGUGGACUG	754	4107	CAGUCCACAUACAGCCGUUC	1073
4107	GUCUUUUUAUUUUUUUCU	755	4107	GUCUUUUUAUUUUUUUCU	755	4125	AGAAAAAUUAAAAAGAC	1074
4125	UCUCUGGAGCCCUCCUCC	756	4125	UCUCUGGAGCCCUCCUCC	756	4143	GGAGGAGGGGCUCCAGAGA	1075
4143	CCCCGGCUGGGCCUCCUUC	757	4143	CCCCGGCUGGGCCUCCUUC	757	4161	GAAGGAGGCCCGCAGCCGGG	1076
4161	CUUCCACUUCUCCCAAGAU	758	4161	CUUCCACUUCUCCCAAGAU	758	4179	AUUCUUGGAGAGUUGGAAG	1077



4179	UGGAAGCCUGAACUGAGGC	759	4179	UGGAAGCCUGAACUGAGGC	759	4197	GCCUCAGUUCAGGCUUCCA	1078
4197	CCUUGUGUGUCAGGCCUC	760	4197	CCUUGUGUGUCAGGCCUC	760	4215	GAGGCCUGACACACAAGG	1079
4215	CUGCCUGCACUCCUGGCC	761	4215	CUGCCUGCACUCCUGGCC	761	4233	GGCCAGGGAGUGCAGGCAG	1080
4233	CUUGCCCGUGUGUGUGA	762	4233	CUUGCCCGUGUGUGUGA	762	4251	UCAGCACACGACGGGCAAG	1081
4251	AAGCAUGUUAAGAACC	763	4251	AAGCAUGUUAAGAACC	763	4269	GGUUCUUGAAACAUGUCUU	1082
4269	CGCAUUCGGGAAGGCA	764	4269	CGCAUUCGGGAAGGCA	764	4287	UGCCUUCUCCGAAUUGCGG	1083
4287	AUGCACGGCCAUGCACAC	765	4287	AUGCACGGCCAUGCACAC	765	4305	GUGUUCAGGCCCGUGCAU	1084
4305	CGGCGGUCACUCUGCCU	766	4305	CGGCGGUCACUCUGCCU	766	4323	AGGCAGAGUACCAAGCCG	1085
4323	UCUGCUGCUGCCCGGGUG	767	4323	UCUGCUGCUGCCCGGGUG	767	4341	CACCCGGGCGAGCAGCAGA	1086
4341	GGGGUGCACUGCCAUUUC	768	4341	GGGGUGCACUGCCAUUUC	768	4359	GAAUUGGCGAGUGCACCCC	1087
4359	CCUCACGUGCAGGACAGCU	769	4359	CCUCACGUGCAGGACAGCU	769	4377	AGCUGUCCUGCACGUGAGG	1088
4377	UCUUGAUUUGGUGGAAAA	770	4377	UCUUGAUUUGGUGGAAAA	770	4395	UUUCCACCCAAAUCAAGA	1089
4395	ACAGGGUGCUAAAGCCAAC	771	4395	ACAGGGUGCUAAAGCCAAC	771	4413	GUUGGCUUJAGCACCCUGU	1090
4413	CCAGCCUUUGGUGCCUGGG	772	4413	CCAGCCUUUGGUGCCUGGG	772	4431	CCCAGGACCCAAAGGCGG	1091
4431	GCAGGUGGAGCUGAAAG	773	4431	GCAGGUGGAGCUGAAAG	773	4449	CUUUUCAGCUCGCCACUCC	1092
4449	GGAUCGAGGCAUUGGGCAU	774	4449	GGAUCGAGGCAUUGGGCAU	774	4467	AUGCCCCAUUGCCUGAUCC	1093
4467	UGUCCUUCACUUGUCCA	775	4467	UGUCCUUCACUUGUCCA	775	4485	UGACAGUUGGAAAGGACA	1094
4485	ACAUCCCCAGAGCCAGCU	776	4485	ACAUCCCCAGAGCCAGCU	776	4503	AGCUGGGCUCUGGGGAUGU	1095
4503	UCUUGCUCUCUUGUACGU	777	4503	UCUUGCUCUCUUGUACGU	777	4521	ACGUCACAAGAGCAAGA	1096
4521	UGCACUGUGAAUCCUGGCA	778	4521	UGCACUGUGAAUCCUGGCA	778	4539	UGCCAGGAUUCACAGUGCA	1097
4539	AAGAAAGCUUGAGUCUCAA	779	4539	AAGAAAGCUUGAGUCUCAA	779	4557	UUGAGACUCAAGCUUUUCU	1098
4557	AGGGUGGCAGGUCACUGUC	780	4557	AGGGUGGCAGGUCACUGUC	780	4575	GACAGUACCUGCCACCCU	1099
4575	CACUGCCGACAUCCUCCC	781	4575	CACUGCCGACAUCCUCCC	781	4593	GGGAGGAGUUGCGGCAGUG	1100
4593	CCCAGCAGAAUGGAGGCAG	782	4593	CCCAGCAGAAUGGAGGCAG	782	4611	CUGCCUCCAUUCUGCUGGG	1101
4611	GGGGACAAGGGAGGACAGUG	783	4611	GGGGACAAGGGAGGACAGUG	783	4629	CACUGCCUCCUUGUCCCC	1102
4629	GGCUAGUGGGGUGAACAGC	784	4629	GGCUAGUGGGGUGAACAGC	784	4647	GCUGUUCACCCACUAGCC	1103
4647	CUGGUGCCAAUAGCCCCA	785	4647	CUGGUGCCAAUAGCCCCA	785	4665	UGGGGCUAUUUGGCACCAG	1104
4665	AGACUGGGCCAGGCAGGU	786	4665	AGACUGGGCCAGGCAGGU	786	4683	ACCUGCCUGGGCCCGAGU	1105
4683	UCUGCAAGGGCCAGAGUG	787	4683	UCUGCAAGGGCCAGAGUG	787	4701	CACUCUGGGCCCUUGCAGA	1106
4701	GAACCGUCCUUUCACACAU	788	4701	GAACCGUCCUUUCACACAU	788	4719	AUGUGUGAAAGGACGGUUC	1107
4719	UCUGGGUGCCUUGAAGGGC	789	4719	UCUGGGUGCCUUGAAGGGC	789	4737	GCCUUCAGGGCACCCAGA	1108
4737	CCCUUCCCUCCCCACUC	790	4737	CCCUUCCCUCCCCACUC	790	4755	GAGUGGGGAGGGGAAGGG	1109
4755	CCUCUAGACAAAGUAGAU	791	4755	CCUCUAGACAAAGUAGAU	791	4773	AUCUACUUGUCUUAAGAGG	1110
4773	UUCUUAAGGGCCCUUUC	792	4773	UUCUUAAGGGCCCUUUC	792	4791	GGAAGGGCCUUGUAAGAA	1111
4791	CUUUGGAACAAGACAGCCU	793	4791	CUUUGGAACAAGACAGCCU	793	4809	AGGUGUCUUGUUCCAAAG	1112
4809	UUCACUUUUUCUGAGUUCU	794	4809	UUCACUUUUUCUGAGUUCU	794	4827	AAGAACUCAGAAAAAGUGAA	1113



4827	UGAAGCAUUAUCAAAGCCCU	795	4827	UGAAGCAUUAUCAAAGCCCU	795	4845	AGGGCUUUGAAAUUGCUUCA	1114
4845	UGCCUCUGUGUAGCGGCC	796	4845	UGCCUCUGUGUAGCGGCC	796	4863	GGCGGCUACACAGAGGCA	1115
4863	CUGAGAGAGAAUAGAGCUG	797	4863	CUGAGAGAGAAUAGAGCUG	797	4881	CAGCUAUUCUCUCUCUCAG	1116
4881	GCCACUGGGCACCUUGCGGA	798	4881	GCCACUGGGCACCUUGCGGA	798	4899	UCGCGAGGUGGCCAGUGGCG	1117
4899	ACAGGUGGGAGGAAAGGGC	799	4899	ACAGGUGGGAGGAAAGGGC	799	4917	GCCUUIUCCUCCACCUUGU	1118
4917	CCUGCGCAGUCCUGGUCCU	800	4917	CCUGCGCAGUCCUGGUCCU	800	4935	AGGACCAGGACUGCGCAGG	1119
4935	UGGUCGACUCUUGAACUG	801	4935	UGGUCGACUCUUGAACUG	801	4953	CAGUUAAGAGUGCAGCCA	1120
4953	GGCGAAUGUCUUAUUUA	802	4953	GGCGAAUGUCUUAUUUA	802	4971	UUAUUUAAGACAUUCGCC	1121
4971	AUUACCGUGAGUGACAUAG	803	4971	AUUACCGUGAGUGACAUAG	803	4989	CUAUGUCACUCACGGUAU	1122
4989	GCCUCAUGUUCUGUGGGG	804	4989	GCCUCAUGUUCUGUGGGG	804	5007	CCCCACAGAACAUAGAGGC	1123
5007	GUCAUCAGGGAGGGUAGG	805	5007	GUCAUCAGGGAGGGUAGG	805	5025	CCUAACCCUCCUGAUGAC	1124
5025	GAAACCCACAAACGGGCC	806	5025	GAAACCCACAAACGGGCC	806	5043	GGCUCGUUUGUGGUUUC	1125
5043	CCUGAAAGCCUACAGUAU	807	5043	CCUGAAAGCCUACAGUAU	807	5061	AUACGUGAGGCUUUCAGGG	1126
5061	UUUCACAGAGCAGCCUGC	808	5061	UUUCACAGAGCAGCCUGC	808	5079	GCAGGCGUGCUCUGUGAAA	1127
5079	CCAUCUUCUCCCGAGGCU	809	5079	CCAUCUUCUCCCGAGGCU	809	5097	AGCCUUGGGGAGAAAGUUG	1128
5097	UGCCCCAGGCGGAGCCCA	810	5097	UGCCCCAGGCGGAGCCCA	810	5115	UGGGUCCCGGCCUGGGGCA	1129
5115	AGUAACCGCGGCGUGUA	811	5115	AGUAACCGCGGCGUGUA	811	5133	UCACAGCCCGCGGUUUCU	1130
5133	ACUCUGGGAGGACCCGG	812	5133	ACUCUGGGAGGACCCGG	812	5151	CCGGUCCUUGCCAGAGU	1131
5151	GGGUCUCCUGGACCUUGAC	813	5151	GGGUCUCCUGGACCUUGAC	813	5169	GUCAAGGUCCAGGAGACCC	1132
5169	CAGAGCAGCUAACUCCGAG	814	5169	CAGAGCAGCUAACUCCGAG	814	5187	CUCGGAGUAGCUUCUCUG	1133
5187	GAGCAGUGGCGAGGUGGC	815	5187	GAGCAGUGGCGAGGUGGC	815	5205	GGCCACCUGCCACUUCUC	1134
5205	CGCCCUAGGCGUUCACGC	816	5205	CGCCCUAGGCGUUCACGC	816	5223	GCUGAAGCCUACAGGGGCG	1135
5223	CCGGAGAGCCACCUUCCC	817	5223	CCGGAGAGCCACCUUCCC	817	5241	GGGAAGGUGGCUUCUCCGG	1136
5241	CGCCCUUACUACCGCCUC	818	5241	CGCCCUUACUACCGCCUC	818	5259	GAGCGGUUAGAAAGGGGCG	1137
5259	CGUGCCAGCAGCCUCCGAC	819	5259	CGUGCCAGCAGCCUCCGAC	819	5277	GUGCGAGGUGCUGGGCAG	1138
5277	CAGGCCUAGCUUUUACGCU	820	5277	CAGGCCUAGCUUUUACGCU	820	5295	AGCGUAAAGCUAGGGCCUG	1139
5295	UCAUCACCUAAACUUGUAC	821	5295	UCAUCACCUAAACUUGUAC	821	5313	GUACAAGUUUAGGUGAUGA	1140
5313	CUUUUUUUUCUGAUAGAA	822	5313	CUUUUUUUUCUGAUAGAA	822	5331	UUCUAUCAGAAAAUAAAG	1141
5331	AUUGGUUCCUCUGGAUCG	823	5331	AUUGGUUCCUCUGGAUCG	823	5349	CGAUCCAGAGGAAACCAUU	1142
5349	GUUUUAGCGGUUCUUAACA	824	5349	GUUUUAGCGGUUCUUAACA	824	5367	UGUAAGAACCAGCAUAAAC	1143
5367	AGCACAUACACCUUUUCCC	825	5367	AGCACAUACACCUUUUCCC	825	5385	GGGAAAGAGGUGAUGUCU	1144
5385	CCCGACGGCUGUGACGCA	826	5385	CCCGACGGCUGUGACGCA	826	5403	UGCGUCACAGCCGUGGGG	1145
5403	AGCGGAGAGGACUAGUCA	827	5403	AGCGGAGAGGACUAGUCA	827	5421	UGACUAGUGCCUCUCCGCU	1146
5421	ACCGACAGCGGCCUUGAAG	828	5421	ACCGACAGCGGCCUUGAAG	828	5439	CUUCAAGGCCGUCUCGGU	1147
5439	GACAGAGCAAAAGCCCCAC	829	5439	GACAGAGCAAAAGCCCCAC	829	5457	GUGGGGCUUUGCUUCUUC	1148
5457	CCCAGGUCCCCCGACUGCC	830	5457	CCCAGGUCCCCCGACUGCC	830	5475	GGCAGUCGGGGGACCUUGG	1149

5475	CUGUCUCCAUAGAGUACUG	831	5475	CUGUCUCCAUAGAGUACUG	831	5493	CAGUACCUCUAGGAGACAG	1150
5493	GGUCCCUUCCUUUGUAA	832	5493	GGUCCCUUCCUUUGUAA	832	5511	UUAACAAAGGAGGGACC	1151
5511	ACGUGAUGUGCCACUAAU	833	5511	ACGUGAUGUGCCACUAAU	833	5529	AUAUAGUGGCACUACACGU	1152
5529	UUUACACGUAUCUCUUGG	834	5529	UUUACACGUAUCUCUUGG	834	5547	CCAAGAGAUACGUGUAAA	1153
5547	GUAUGCAUCUUUAUAGAC	835	5547	GUAUGCAUCUUUAUAGAC	835	5565	GUCUAUAAAAGAUCAUAC	1154
5565	CGCUCUUUUAAGUGGCG	836	5565	CGCUCUUUUAAGUGGCG	836	5583	CGCCACUUAGAAAAAGCG	1155
5583	GUGUGCAUAGCUCUCCG	837	5583	GUGUGCAUAGCUCUCCG	837	5601	GGCAGGACGCUAUGCACAC	1158
5601	CCUGCCUCCGGGCGCUGU	838	5601	CCUGCCUCCGGGCGCUGU	838	5619	ACAGGCCCGAGGGCAGG	1157
5619	UGGUGGCUCCCGCUCUGU	839	5619	UGGUGGCUCCCGCUCUGU	839	5637	AGCAGAGGGGAGCCACCA	1158
5637	UUCUGGGGUCAGUGCAU	840	5637	UUCUGGGGUCAGUGCAU	840	5655	AUGCACUGGACCCCGAGAA	1159
5655	UUUUGUUUCUGUAUAGAU	841	5655	UUUUGUUUCUGUAUAGAU	841	5673	AUCAUAUACAGAAACAAA	1160
5673	UUCUCUGUGGUUUUUUUG	842	5673	UUCUCUGUGGUUUUUUUG	842	5691	CAAAAAACACACAGAGAA	1161
5691	GAUCCAAUUCUGUCCUCU	843	5691	GAUCCAAUUCUGUCCUCU	843	5709	AGAGGACAGAUUUGGAUUC	1162
5709	UGUAGUAUUUUUUAUAA	844	5709	UGUAGUAUUUUUUAUAA	844	5727	UUUUUUAAAAAUACUACA	1163
5724	AUAAUACAGUGUUUACAUU	845	5724	AUAAUACAGUGUUUACAUU	845	5742	AUUGUAAACACUGAUUUU	1164

**HSA131467 (b2a2)**

Pos	Target Sequence	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
281	UGACCAUCAUAAGGAAGA	1165	281	UGACCAUCAUAAGGAAGA	1165	299	UCUCCUUUAUUGAUGGUC	1183
282	GACCAUCAUAAGGAAGA	1166	282	GACCAUCAUAAGGAAGA	1166	300	UUCUCCUUUAUUGAUGGUC	1184
283	ACCAUCAUAAGGAAGA	1167	283	ACCAUCAUAAGGAAGA	1167	301	CUUCUCCUUUAUUGAUGGUC	1185
284	CCAUCAUAAGGAAGA	1168	284	CCAUCAUAAGGAAGA	1168	302	GCUUCUCCUUUAUUGAUGG	1186
285	CAUCAUAAGGAAGA	1169	285	CAUCAUAAGGAAGA	1169	303	GGCUUCUCCUUUAUUGAUG	1187
286	AUCAUAAGGAAGA	1170	286	AUCAUAAGGAAGA	1170	304	GGGUUCUCCUUUAUUGAU	1188
287	UCAUAAGGAAGA	1171	287	UCAUAAGGAAGA	1171	305	AGGGCUUCUCCUUUAUUG	1189
288	CAUAAGGAAGA	1172	288	CAUAAGGAAGA	1172	306	AAGGGCUUCUCCUUUAUUG	1190
289	AUAAGGAAGA	1173	289	AUAAGGAAGA	1173	307	GAAGGGCUUCUCCUUUAU	1191
290	AUAAGGAAGA	1174	290	AUAAGGAAGA	1174	308	UGAAGGGCUUCUCCUUUA	1192
291	UAAGGAAGA	1175	291	UAAGGAAGA	1175	309	CUGAAGGGCUUCUCCUUUA	1193
292	AAGGAAGA	1176	292	AAGGAAGA	1176	310	GCUGAAGGGCUUCUCCUU	1194
293	AGGAAGA	1177	293	AGGAAGA	1177	311	CGCUGAAGGGCUUCUCCU	1195
294	GGAAGA	1178	294	GGAAGA	1178	312	CCGCUGAAGGGCUUCUCC	1196
295	GAAGA	1179	295	GAAGA	1179	313	GCCGUGAAGGGCUUCUCC	1197
296	AAGA	1180	296	AAGA	1180	314	GGCCGUGAAGGGCUUCU	1198
297	AGA	1181	297	AGA	1181	315	UGCCGUGAAGGGCUUCU	1199
298	GA	1182	298	GA	1182	316	CUGGCCGUGAAGGGCUUC	1200

## HSA131466 (b3a2)

Pos	Target Sequence	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
358	GAUUUAAGCAGAGUUCAAA	1201	356	GAUUUAAGCAGAGUUCAAA	1201	374	UUUGAACUCUGCUUAAAUC	1219
357	AUUUAAGCAGAGUUCAAA	1202	357	AUUUAAGCAGAGUUCAAA	1202	375	UUUUGAACUCUGCUUAAA	1220
358	UUUAAGCAGAGUUCAAAAG	1203	358	UUUAAGCAGAGUUCAAAAG	1203	376	CUUUUGAACUCUGCUUAAA	1221
359	UUUAAGCAGAGUUCAAAAGC	1204	359	UUUAAGCAGAGUUCAAAAGC	1204	377	GCUUUUUGAACUCUGCUUAA	1222
360	UAAGCAGAGUUCAAAAGCC	1205	360	UAAGCAGAGUUCAAAAGCC	1205	378	GGCUUUUGAACUCUGCUUA	1223
361	AAGCAGAGUUCAAAAGCCC	1206	361	AAGCAGAGUUCAAAAGCCC	1206	379	GGGCUUUUGAACUCUGCUU	1224
362	AGCAGAGUUCAAAAGCCCU	1207	362	AGCAGAGUUCAAAAGCCCU	1207	380	AGGGCUUUUGAACUCUGCU	1225
363	GCAGAGUUCAAAAGCCCUU	1208	363	GCAGAGUUCAAAAGCCCUU	1208	381	AAGGGCUUUUGAACUCUGC	1226
364	CAGAGUUCAAAAGCCCUUC	1209	364	CAGAGUUCAAAAGCCCUUC	1209	382	GAAGGGCUUUUGAACUCUG	1227
365	AGAGUUCAAAAGCCCUUCA	1210	365	AGAGUUCAAAAGCCCUUCA	1210	383	UGAAGGGCUUUUGAACUCU	1228
366	GAGUUCAAAAGCCCUUCAG	1211	366	GAGUUCAAAAGCCCUUCAG	1211	384	CUAAGGGCUUUUGAACUC	1229
367	AGUUCAAAAGCCCUUCAGC	1212	367	AGUUCAAAAGCCCUUCAGC	1212	385	GCUGAAGGGCUUUUGAACU	1230
368	GUUCAAAAGCCCUUCAGCG	1213	368	GUUCAAAAGCCCUUCAGCG	1213	386	CGCUGAAGGGCUUUUGAAC	1231
369	UUCAAAAGCCCUUCAGCGG	1214	369	UUCAAAAGCCCUUCAGCGG	1214	387	CCGCUGAAGGGCUUUUGAA	1232
370	UCAAAGCCCUUCAGCGGC	1215	370	UCAAAGCCCUUCAGCGGC	1215	388	GCCGCUGAAGGGCUUUUGA	1233
371	CAAAAGCCCUUCAGCGGCC	1216	371	CAAAAGCCCUUCAGCGGCC	1216	389	GGCCGCUGAAGGGCUUUUG	1234
372	AAAAGCCCUUCAGCGGCCA	1217	372	AAAAGCCCUUCAGCGGCCA	1217	390	UGGCCGCUGAAGGGCUUUU	1235
373	AAAGCCCUUCAGCGGCCAG	1218	373	AAAGCCCUUCAGCGGCCAG	1218	391	CUGGCCGCUGAAGGGCUUU	1236

## NM\_004449|ERG2

Pos	Target Sequence	Seq ID	UPos	Upper seq	Seq ID	LPos	Lower seq	Seq ID
1	GUCCGCGGUGUCCGCGCC	1237	1	GUCCGCGGUGUCCGCGCC	1237	23	GGCGCGGACACGCGCGGAC	1413
19	CCGCGUGUGCCAGCGCGCG	1238	19	CCGCGUGUGCCAGCGCGCG	1238	41	CGCGCGUGGACACACGCGG	1414
37	GUGCCUUGGCGUGCGCGCG	1239	37	GUGCCUUGGCGUGCGCGCG	1239	59	GGCGCGCACGGCCAAAGGCAC	1415
55	CCGAGCCGGGUGCGACUAA	1240	55	CCGAGCCGGGUGCGACUAA	1240	77	UUAGUGCGACCCCGGCGCGG	1416
73	ACUCCUUGCGCGCGACGG	1241	73	ACUCCUUGCGCGCGACGG	1241	95	CCGUGCGCGCCGAGGGAGU	1417
91	GCGGCGCUAACCCUCUCGGU	1242	91	GCGGCGCUAACCCUCUCGGU	1242	113	CCGAGAGGUGUAGCGCGCG	1418
109	UUUUCCAGGAUUCUUUGGA	1243	109	UUUUCCAGGAUUCUUUGGA	1243	131	UCCAAAGAUCCUGGAAUAA	1419
127	AGACCGAGGAAAGCCGUG	1244	127	AGACCGAGGAAAGCCGUG	1244	149	CACGGCUUUUCCUGCGGUCU	1420
145	GUUGACCAAAAGCAAGACA	1245	145	GUUGACCAAAAGCAAGACA	1245	167	UGUCUUGCUUUUUGGUCAAC	1421
163	AAUAGACUCACAGAGAAAA	1246	163	AAUAGACUCACAGAGAAAA	1246	185	UUUUCUCUGAGUGCAUUU	1422
181	AAAGUAGCAGAACCAAGG	1247	181	AAAGUAGCAGAACCAAGG	1247	203	CCUUGGUUUCUGCCCAUUU	1423

199	GGCAACUAAAGCCGUCAGG	1248	199	GGCAACUAAAGCCGUCAGG	1248	221	CCUGACGGCUUUAAGUUGCC	1424
217	GUUCUGAACAGCUGGUAGA	1249	217	GUUCUGAACAGCUGGUAGA	1249	239	UCUACCAGCUGUUCAGAAC	1425
235	AUGGGCUGGCUUACUGAAG	1250	235	AUGGGCUGGCUUACUGAAG	1250	257	CUUCAGUAAAGCCAGCCCAU	1426
253	GGACAUGAUUCAGACUGUC	1251	253	GGACAUGAUUCAGACUGUC	1251	275	GACAGUCUGAAUUAUUGUCC	1427
271	CCCGGACCCAGCAGCUCAU	1252	271	CCCGGACCCAGCAGCUCAU	1252	293	AUGAGCUGCUGGGUCCGGG	1428
289	UAUCAAGGAAGCCUUAUCA	1253	289	UAUCAAGGAAGCCUUAUCA	1253	311	UGAAUAGGCUUCCUUGAUA	1429
307	AGUUGUGAGUGAGGACCAG	1254	307	AGUUGUGAGUGAGGACCAG	1254	329	CUGGUCCUCACUCACAACU	1430
325	GUCGUUGUUGAGUGUGCC	1255	325	GUCGUUGUUGAGUGUGCC	1255	347	GGCACACUCAACAACGAC	1431
343	CUACGGGAACGCCACACCU	1256	343	CUACGGGAACGCCACACCU	1256	365	CAGGUGUGGGCUUCCGUAG	1432
361	GGCUAAGACAGAGUAGACC	1257	361	GGCUAAGACAGAGUAGACC	1257	383	GUCAUCUCUGUCUUAGGCC	1433
379	CGCGUCCUCCUCCAGCGAC	1258	379	CGCGUCCUCCUCCAGCGAC	1258	401	GUCCGUGBAGBAGGAGCGG	1434
397	CUAUGGACAGACUUCCAA	1259	397	CUAUGGACAGACUUCCAA	1259	419	CUUGGAAGUCUGUCCAUAG	1435
415	GAUGAGCCACCGCUGCCU	1260	415	GAUGAGCCACCGCUGCCU	1260	437	AGGACGCGUGGGCUCAC	1436
433	UCAGCAGGGAUUGGCUGUC	1261	433	UCAGCAGGGAUUGGCUGUC	1261	455	AGACAGCAAUCCUGCUGA	1437
451	UCAACCCCGGAGGCGGUC	1262	451	UCAACCCCGGAGGCGGUC	1262	473	GACCCUGGCGUGGGGUUGA	1438
469	CACCAUCAAAUUGGAUUG	1263	469	CACCAUCAAAUUGGAUUG	1263	491	ACAUUCCAUUUUGAUGGUG	1439
487	UAACCCUAGCCAGGUGAAU	1264	487	UAACCCUAGCCAGGUGAAU	1264	509	AUUCACCCUGGCUAGGGUUA	1440
505	UGGCUCAAGGAACUCUCUC	1265	505	UGGCUCAAGGAACUCUCUC	1265	527	AGGAGAUUCCUUGAGCCA	1441
523	UGAUGAAUGCAGUGUGGCC	1266	523	UGAUGAAUGCAGUGUGGCC	1266	545	GGCCACAGUCGAUUAUCA	1442
541	CAAAGGCGGGAAGUUGGUG	1267	541	CAAAGGCGGGAAGUUGGUG	1267	563	CACCAUUCUCCCGCCUUG	1443
559	GGCAGCCCGACACACCGU	1268	559	GGCAGCCCGACACACCGU	1268	581	AACGGUGUCUGGGCUGCCC	1444
577	UGGGAUGAACUACGGCAGC	1269	577	UGGGAUGAACUACGGCAGC	1269	599	GCUGCCGUAUUAUCAUCCA	1445
595	CUACAUGGAGGAGAGCAG	1270	595	CUACAUGGAGGAGAGCAG	1270	617	GUGCUUCUCCUCCAUUGUAG	1446
613	CAUGCCACCCCAACCAUG	1271	613	CAUGCCACCCCAACCAUG	1271	635	CAUGUUUGGGGUGGGCAUG	1447
631	GACCACGAACGAGCGCAGA	1272	631	GACCACGAACGAGCGCAGA	1272	653	UCUGCGCUCGUUCGUGGUC	1448
649	AGUUAUCGUGCCAGCAGAU	1273	649	AGUUAUCGUGCCAGCAGAU	1273	671	AUCUGCUGGCGACGAUAAU	1449
667	UCCUACGCUAUGGAGUACA	1274	667	UCCUACGCUAUGGAGUACA	1274	689	UGUACUCCAUAGCGUAGGA	1450
685	AGACCAUGUGCGGCAGUGG	1275	685	AGACCAUGUGCGGCAGUGG	1275	707	CCACUGCCGCACAUUGGUCU	1451
703	GCUGGAGUGGGCGGUGAAA	1276	703	GCUGGAGUGGGCGGUGAAA	1276	725	UUUACCCGCCACUCCAGC	1452
721	AGAAUUGGCCUUCACAGAC	1277	721	AGAAUUGGCCUUCACAGAC	1277	743	GUCUGGAAGGCCAUUAUUCU	1453
739	CGUCAACAUCUUGUUAUUC	1278	739	CGUCAACAUCUUGUUAUUC	1278	761	GAAUACAAGGAUUGUAGCG	1454
757	CCAGAACAUCCGAUGGGAAG	1279	757	CCAGAACAUCCGAUGGGAAG	1279	779	CUUCCCAUCGAUUGUUGG	1455
775	GGAACUGUGCAAGAUAGACC	1280	775	GGAACUGUGCAAGAUAGACC	1280	797	GGUCAUCUUGCACAGUUC	1456
793	CAAGGACGACUUCAGAGG	1281	793	CAAGGACGACUUCAGAGG	1281	815	CCUCUGGAAGUCGUCUUG	1457
811	GCUCACCCCGACGCUACAAC	1282	811	GCUCACCCCGACGCUACAAC	1282	833	GUUGUAGCUGGGGUGAGC	1458
829	CGCCGACAUCCUUCUCUCA	1283	829	CGCCGACAUCCUUCUCUCA	1283	851	UGAGAGAAGGAUUGCGGCG	1459

847	ACAUCUCCACUACCUACAGA	1284	847	ACAUCUCCACUACCUACAGA	1284	869	UCUGAGGUAGUGGAGAUUGU	1460
865	AGAGACUCCUUCUCCACAU	1285	865	AGAGACUCCUUCUCCACAU	1285	887	AUGUGGAAGAGGAGUCUCU	1461
883	UUUGACUUCAGAUAGUUGU	1286	883	UUUGACUUCAGAUAGUUGU	1286	905	AACAUCUUCUGAAGUCAAA	1462
901	UGAUAAAGCCUUAACAAAC	1287	901	UGAUAAAGCCUUAACAAAC	1287	923	GUUUUGUAAAGGCUUUUAUCA	1463
919	CUCUCACGGUUAUUGCAU	1288	919	CUCUCACGGUUAUUGCAU	1288	941	UAGCAUUAACCGUUGGAGAG	1464
937	UGCUAGAAACACAGAUUUA	1289	937	UGCUAGAAACACAGAUUUA	1289	959	UAAUCUGUUCUUCUAGCA	1465
955	ACCAUUGAGCCCCCAGG	1290	955	ACCAUUGAGCCCCCAGG	1290	977	CCUGGGGGGCUUUAUGGU	1466
973	GAGAUACGCCUGGACCGGU	1291	973	GAGAUACGCCUGGACCGGU	1291	995	ACCGGUCCAGGCUUGAUCUC	1467
991	UCACGGCCACCCGACGCC	1292	991	UCACGGCCACCCGACGCC	1292	1013	GGGCGUGGGUGGCCGUGA	1468
1009	CCAGUCGAAAGCUGCUCAA	1293	1009	CCAGUCGAAAGCUGCUCAA	1293	1031	UUGAGCAGCUUUCGACUGG	1469
1027	ACCAUCUCCUUCACAGUG	1294	1027	ACCAUCUCCUUCACAGUG	1294	1049	CACUGUGGAAGGAGAUUGU	1470
1045	GCCCAAAACUGAAGACCAG	1295	1045	GCCCAAAACUGAAGACCAG	1295	1067	CUGGCUUUCAGUUUUUGGC	1471
1063	GCUCUCCUAGUAGAUCCU	1296	1063	GCUCUCCUAGUAGAUCCU	1296	1085	AGGAUCUAACUGAGGACGC	1472
1081	UUUAUCAGAUUCUUGGACCA	1297	1081	UUUAUCAGAUUCUUGGACCA	1297	1103	UGGUCCAAGAAUCUGAUAA	1473
1099	AACAAGUAGCCGCUUGCA	1298	1099	AACAAGUAGCCGCUUGCA	1298	1121	UGCAAGGGCGCUACUUGUU	1474
1117	AAUCCAGGCAGUGGCCAG	1299	1117	AAUCCAGGCAGUGGCCAG	1299	1139	CUGGCCACUGCCUGGAUUU	1475
1135	GAUCCAGCUUUGGCAGUUC	1300	1135	GAUCCAGCUUUGGCAGUUC	1300	1157	GAACUGCCAAAGCUGGAGUC	1476
1153	CCUCCUGGAGCUCCUGUGG	1301	1153	CCUCCUGGAGCUCCUGUGG	1301	1175	CGACAGGAGCUCGAGG	1477
1171	GGACAGCUCCAACUCCAGC	1302	1171	GGACAGCUCCAACUCCAGC	1302	1193	GCUGGAGUUGGAGCUGUCC	1478
1189	CUGCAUCACUUGGGAAGGC	1303	1189	CUGCAUCACUUGGGAAGGC	1303	1211	GCCUCCAGGUGUGAUCAG	1479
1207	CACCAACGGGAGUUAAG	1304	1207	CACCAACGGGAGUUAAG	1304	1229	CUUGAACUCCCGUUGGUG	1480
1225	GAUGACGGAUCCCGAGGAG	1305	1225	GAUGACGGAUCCCGAGGAG	1305	1247	CUCGUCGGGAUCCGUAUC	1481
1243	GGUGCCCGGCGCUGGGGA	1306	1243	GGUGCCCGGCGCUGGGGA	1306	1265	UCCCAAGCGCCGGGCCACC	1482
1261	AGAGCGGAAGAGCAAAACC	1307	1261	AGAGCGGAAGAGCAAAACC	1307	1283	GGGUUUGCUCUUCGCGUCU	1483
1279	CAACAUGAACUACGAUAG	1308	1279	CAACAUGAACUACGAUAG	1308	1301	CUUAUCGUAGUUAUGUUG	1484
1297	GCUCAGCCGCGCCUCCGU	1309	1297	GCUCAGCCGCGCCUCCGU	1309	1319	ACGGAGGGCGCGGCUAGGC	1485
1315	UUACUACUAGACAAGAAC	1310	1315	UUACUACUAGACAAGAAC	1310	1337	GUUCUUGUACUAGUAGUAA	1486
1333	CAUCAUGACCAAGGUCCAU	1311	1333	CAUCAUGACCAAGGUCCAU	1311	1355	AUGGACCUUGGUCAUGAU	1487
1351	UGGGAAGCGUACGCCUAC	1312	1351	UGGGAAGCGUACGCCUAC	1312	1373	GUAGCGUAGCGCUUCCCA	1488
1369	CAAGUUCGACUUCACGGG	1313	1369	CAAGUUCGACUUCACGGG	1313	1391	CCGUGGAAAGUCGAACUUG	1489
1387	GAUCGCCAGGCCUCCAG	1314	1387	GAUCGCCAGGCCUCCAG	1314	1409	CUGGAGGGCCUGGGCGAUC	1490
1405	GCCCCACCCCGGAGUCA	1315	1405	GCCCCACCCCGGAGUCA	1315	1427	UGACUCCGGGGGUGGGGC	1491
1423	AUCUCUGUACAAGUACCCC	1316	1423	AUCUCUGUACAAGUACCCC	1316	1445	GGGUAACUUGUACAGAU	1492
1441	CUCAGACCUCCCGUACUAG	1317	1441	CUCAGACCUCCCGUACUAG	1317	1463	CAUGUACGGGAGGUCUGAG	1493
1459	GGGCUCCUUAUCAGGCCAC	1318	1459	GGGCUCCUUAUCAGGCCAC	1318	1481	GUGGGCGUGAUGGAGCCC	1494
1477	CCCACAGAAGAUAGAUCUUU	1319	1477	CCCACAGAAGAUAGAUCUUU	1319	1499	AAAGUUAUCUUCUGUGGG	1495

1495	UGUGGGCCCCACCCUCCA	1320	1495	UGUGGGCCCCACCCUCCA	1320	1517	UGAGGGUGGGCGCCACA	1496
1513	AGCCUCCCCGUGACAUCU	1321	1513	AGCCUCCCCGUGACAUCU	1321	1535	AGAUUCACGGGAGGGCU	1497
1531	UUCAGUUUUUGUGGCC	1322	1531	UUCAGUUUUUGUGGCC	1322	1553	GGCAGCAAAAACUIGGAA	1498
1549	CCCAAAACCAUACUGGAU	1323	1549	CCCAAAACCAUACUGGAU	1323	1571	AUUCAGAUUGGUUUGG	1499
1567	UUCACCAACUGGGGUUA	1324	1567	UUCACCAACUGGGGUUA	1324	1589	UAUACCCCAGUUGGUGAA	1500
1585	UAACCCCAACUAGGCUC	1325	1585	UAACCCCAACUAGGCUC	1325	1607	GAGCCUAGUUGGGGUUA	1501
1603	CCCACCAACCAUAGCCU	1326	1603	CCCACCAACCAUAGCCU	1326	1625	AGCAUAGGCGUGGGG	1502
1621	UUCUCAUCUGGCACUAC	1327	1621	UUCUCAUCUGGCACUAC	1327	1643	GUAGUGCCAGAGAGAA	1503
1639	CUACUAAAGACUGGCGGA	1328	1639	CUACUAAAGACUGGCGGA	1328	1661	UCCGCCAGGUCUUUAGUAG	1504
1657	AGCUUUUCCCCAUCAGCGU	1329	1657	AGCUUUUCCCCAUCAGCGU	1329	1679	AGCGUAGUGGAAAAGCCU	1505
1675	UGCAUUCACAGCCCAUCG	1330	1675	UGCAUUCACAGCCCAUCG	1330	1697	CGAUUGGCGUGGUAAGCA	1506
1693	GCCACAAACUUAUCGGAG	1331	1693	GCCACAAACUUAUCGGAG	1331	1715	CUCCGAUAGAGUUGGGC	1507
1711	GAACAUGAAUCAAAGUGC	1332	1711	GAACAUGAAUCAAAGUGC	1332	1733	GCACUUUUGAUUCAUUGUC	1508
1729	CCUCAAGAGGAUUGAAAA	1333	1729	CCUCAAGAGGAUUGAAAA	1333	1751	UUUUCAUUCUCUUGAGG	1509
1747	AAGCUUACUGGGGUGGG	1334	1747	AAGCUUACUGGGGUGGG	1334	1769	CCAGCCCCAGUAAAAGCUU	1510
1765	GGAAGGAAGCGGGGAAGA	1335	1765	GGAAGGAAGCGGGGAAGA	1335	1787	UCUUCCCCGCUUCCUCC	1511
1783	AGAUCCAAAGACUCUUGGG	1336	1783	AGAUCCAAAGACUCUUGGG	1336	1805	CCCAAGAGUCUUUGGACU	1512
1801	GAGGGAGUUAUCUGAAGUCU	1337	1801	GAGGGAGUUAUCUGAAGUCU	1337	1823	AGACUAGUAACUCCUCC	1513
1819	UAUCUACAGAAUAGGAG	1338	1819	UAUCUACAGAAUAGGAG	1338	1841	CUCCUACUUUCUGUAGUAA	1514
1837	GGAUGCUAAAAUGUCACG	1339	1837	GGAUGCUAAAAUGUCACG	1339	1859	CGUGACAUUUUJAGCAUCC	1515
1855	GAUAUUGGACAUACAUCU	1340	1855	GAUAUUGGACAUACAUCU	1340	1877	AGAUAGUAGUCCAUUAUC	1516
1873	UGUGGACUGACCUUGUAAA	1341	1873	UGUGGACUGACCUUGUAAA	1341	1895	UUUACAAGGUCAGUCCACA	1517
1891	AAGACAGUUAUGUAGAAG	1342	1891	AAGACAGUUAUGUAGAAG	1342	1913	CUUCUACAUACACUGUCUU	1518
1909	GCAUGAGUCUUAAGGACA	1343	1909	GCAUGAGUCUUAAGGACA	1343	1931	UGUCCUUUAGACUUCUAGC	1519
1927	AAUGGCCAAAGAAUGUG	1344	1927	AAUGGCCAAAGAAUGUG	1344	1949	CCACUUUCUUUUGGCACUUU	1520
1945	GUCUUAAGAAUUAUAAA	1345	1945	GUCUUAAGAAUUAUAAA	1345	1967	UUUUAACAUUUUCUUAAGAC	1521
1963	ACUUAAGAGUAGAGUUUGA	1346	1963	ACUUAAGAGUAGAGUUUGA	1346	1985	UCAACUCUACUCUUAAGU	1522
1981	AUCCCAUUAUGCAAACU	1347	1981	AUCCCAUUAUGCAAACU	1347	2003	AGUUUGCAUUAAGUGGGAUU	1523
1999	UGGGAUGAAACUAAAGCAA	1348	1999	UGGGAUGAAACUAAAGCAA	1348	2021	UUUCUUUAGUUUUCAUCCCA	1524
2017	AUAGAAACACACAGUUUU	1349	2017	AUAGAAACACACAGUUUU	1349	2039	AAAACUGUGUUGUUUUCUUA	1525
2035	UGACCUAACAACCGUUUA	1350	2035	UGACCUAACAACCGUUUA	1350	2057	UAAACGGUAUGUUAGGUCA	1526
2053	AUAUUGCCAUUUUAAGGAA	1351	2053	AUAUUGCCAUUUUAAGGAA	1351	2075	UUCCUUAAAUGGCAUUAU	1527
2071	AAACUACCGUUAUUUAAA	1352	2071	AAACUACCGUUAUUUAAA	1352	2093	UUUUAAAUAACAGGUAGUUU	1528
2089	AAUAGUUUCAUAUCAA AAA	1353	2089	AAUAGUUUCAUAUCAA AAA	1353	2111	UUUUUGAUUGAAACUUAU	1529
2107	ACAAGAGAAAAGACACGAG	1354	2107	ACAAGAGAAAAGACACGAG	1354	2129	CUCGUGUCUUUUUCUCUUGU	1530
2125	GAGAGACUGUGGCCCAUCA	1355	2125	GAGAGACUGUGGCCCAUCA	1355	2147	UGAUGGGCCACAGUCUCUC	1531

2143	AACAGACGUUGAUUGCAA	1356	2143	AACAGACGUUGAUUGCAA	1356	2165	UUGCAUAUACAACGUCUGUU	1532
2161	ACUGCAUGGCAUGUGCUGU	1357	2161	ACUGCAUGGCAUGUGCUGU	1357	2183	ACAGCAUAGCCCAUGCAGU	1533
2179	UUUUGGUUGAAAUCAAAUA	1358	2179	UUUUGGUUGAAAUCAAAUA	1358	2201	UAUUUGAUUUACAACCAAAA	1534
2197	ACAUUCCGUUUUGAGGACA	1359	2197	ACAUUCCGUUUUGAGGACA	1359	2219	UGUCCAUAACAACGGAUUGU	1535
2215	AGCUGUCAGCUUUCUCAA	1360	2215	AGCUGUCAGCUUUCUCAA	1360	2237	UUUGAGAAAGCUGACAGCU	1536
2233	ACUGUGAAGAUAGACCCAAA	1361	2233	ACUGUGAAGAUAGACCCAAA	1361	2255	UUUGGUGCAUUCUACAGU	1537
2251	AGUUUCCCAACUCCUUACA	1362	2251	AGUUUCCCAACUCCUUACA	1362	2273	UUUGAAGGAGUUGGAAACU	1538
2269	AGUAUUACCGGACUAUGA	1363	2269	AGUAUUACCGGACUAUGA	1363	2281	UCAUAGUCCCGUAUAUCU	1539
2287	AACUAAAAGGUGGACUGA	1364	2287	AACUAAAAGGUGGACUGA	1364	2309	UCAGUCCCAACCUUUUAGUU	1540
2305	AGGAUGUGUAUAGAGUGAG	1365	2305	AGGAUGUGUAUAGAGUGAG	1365	2327	CUCACUGUAUACACAUCUU	1541
2323	GCGUGUGAUUUGAGACAGA	1366	2323	GCGUGUGAUUUGAGACAGA	1366	2345	UCUGUCUAACAUAACACACGC	1542
2341	AGGGUGUGAAGAGGAGGAG	1367	2341	AGGGUGUGAAGAGGAGGAG	1367	2363	CUCUCCUUCUUCACCCCU	1543
2359	GGAAGAGGCAGAGAGGAG	1368	2359	GGAAGAGGCAGAGAGGAG	1368	2381	CUCUUCUCUGCCUCUUC	1544
2377	GGAGACCAGGCUUGGAAAG	1369	2377	GGAGACCAGGCUUGGAAAG	1369	2399	CUUCCCAAGCCUGGUCUCC	1545
2395	GAACUUCUCAAAGCAUUA	1370	2395	GAACUUCUCAAAGCAUUA	1370	2417	UCUAUGCUUGAGAGUUC	1546
2413	AAGACUGGACUCAGGACAU	1371	2413	AAGACUGGACUCAGGACAU	1371	2435	AUGUCCUGAGUCCAGUCUU	1547
2431	UUUGGGACUUGUACAUA	1372	2431	UUUGGGACUUGUACAUA	1372	2453	AUUGUACACAGUCCCAAAA	1548
2449	UGAUUUUUGGAGACUCGAG	1373	2449	UGAUUUUUGGAGACUCGAG	1373	2471	CUCGAGUCUCCAUACUA	1549
2467	GGUUAUGCAGUCAGUGU	1374	2467	GGUUAUGCAGUCAGUGU	1374	2489	ACACUGACUGCAUGAACCC	1550
2485	UUUAACCAACCCAGUGUU	1375	2485	UUUAACCAACCCAGUGUU	1375	2507	AACACUGGGUUUGGUUAA	1551
2503	UAGGAGAAAGGACACAGCG	1376	2503	UAGGAGAAAGGACACAGCG	1376	2525	CGCUGUGUCCUUCUCCUA	1552
2521	GUAAUGGAGAAAGGGAAGU	1377	2521	GUAAUGGAGAAAGGGAAGU	1377	2543	ACUUCUUCUUCUCCAUUAC	1553
2539	UAGUAGAAUUCAGAAACAA	1378	2539	UAGUAGAAUUCAGAAACAA	1378	2561	UUGUUUCUGAAUUCUACUA	1554
2557	AAAUGCGCAUCUCUUCU	1379	2557	AAAUGCGCAUCUCUUCU	1379	2579	AGAAAGAGAUUGCGCAUUUU	1555
2575	UUUGUUUGUCAAUAGAAA	1380	2575	UUUGUUUGUCAAUAGAAA	1380	2597	UUUUCAUUUGACAAACAAA	1556
2593	AUUUUAACUGGAUUUGUCU	1381	2593	AUUUUAACUGGAUUUGUCU	1381	2615	AGACAAUUCAGAUUAAAAU	1557
2611	UGAUUUUUAAGAGAAACAU	1382	2611	UGAUUUUUAAGAGAAACAU	1382	2633	AUGUUUCUCUUAUAAUUA	1558
2629	UUCAGGACCCUACAUAUAU	1383	2629	UUCAGGACCCUACAUAUAU	1383	2651	AUAUUGAUAGGAGUCCUGAA	1559
2647	UGUGGGGCUUUGUUCUCC	1384	2647	UGUGGGGCUUUGUUCUCC	1384	2669	GGAGAACAAAGCCCCACA	1560
2665	CACAGGGUCAGGUAAGAGA	1385	2665	CACAGGGUCAGGUAAGAGA	1385	2687	UCUCUUAACCUAGACCCUGUG	1561
2683	AUGGCCUUCUUGGCUGCCA	1386	2683	AUGGCCUUCUUGGCUGCCA	1386	2705	UGGACGCAAGAGGCCAU	1562
2701	ACAUACAGAAUUCACGCAG	1387	2701	ACAUACAGAAUUCACGCAG	1387	2723	CUGCGUAUUCUUGAUUGU	1563
2719	GGCAUUUUGGUAAGCGGCG	1388	2719	GGCAUUUUGGUAAGCGGCG	1388	2741	GCCGCCUAGCCCAAAUJGCC	1564
2737	CCUCCAGUUUUCUUGAG	1389	2737	CCUCCAGUUUUCUUGAG	1389	2759	CUCAAAGGAAACUUGGAGG	1565
2755	GUCGCGAACGCUUGUCGUU	1390	2755	GUCGCGAACGCUUGUCGUU	1390	2777	AACGCACAGCGUUCGCGAC	1566
2773	UUGUCAGAAUGAAGUAUAC	1391	2773	UUGUCAGAAUGAAGUAUAC	1391	2795	GUUAUUAUUAUUCUGACAA	1567



2791	CAAGUCAUUGUUUUUCCCC	1392	2791	CAAGUCAUUGUUUUUCCCC	1392	2813	GGGAAAAACAUAUGACUUG	1568
2809	CCUUUUUAUAUAUAUAUA	1393	2809	CCUUUUUAUAUAUAUAUA	1393	2831	UAUAUAUAUAUAUAUAUA	1569
2827	AUAUAACUAUGCAUUUAU	1394	2827	AUAUAACUAUGCAUUUAU	1394	2849	AUAAUUGCAUAAGUUUAU	1570
2845	UACACUACGAGUUGAUCUC	1395	2845	UACACUACGAGUUGAUCUC	1395	2867	GAGAUCAACUCUGUAGUGUA	1571
2863	CGGCCAGCCAAAGACACAC	1396	2863	CGGCCAGCCAAAGACACAC	1396	2885	GUGUGUCUUUUGGCUUGCCG	1572
2881	CGACAAAGAGACAAUCGA	1397	2881	CGACAAAGAGACAAUCGA	1397	2903	UCGAUUGUCUUUUUGUGG	1573
2899	AUAUAUUGGCCUUGAAU	1398	2899	AUAUAUUGGCCUUGAAU	1398	2921	AUAUAGGCCACCAUUUAU	1574
2917	UUUUAACUCUGUAUGCUUA	1399	2917	UUUUAACUCUGUAUGCUUA	1399	2939	UAAGCAUACAGAUUAAAA	1575
2935	AAUGUUUACAUAUUGAAGU	1400	2935	AAUGUUUACAUAUUGAAGU	1400	2957	ACUUCAUUAUUGUAACAUU	1576
2953	UUUAUAGUUCUUGAAGUC	1401	2953	UUUAUAGUUCUUGAAGUC	1401	2975	GCAUUCUAAGAACUAUAA	1577
2971	CAGAAUGUAUGUAUAAAA	1402	2971	CAGAAUGUAUGUAUAAAA	1402	2993	UUUUUAUAUAUAUAUUCUG	1578
2989	AUAAGCUUGGCCUAGCAUG	1403	2989	AUAAGCUUGGCCUAGCAUG	1403	3011	CAUGCAGGCCAAGCUUAU	1579
3007	GGCAAAUCAGAUUAUACA	1404	3007	GGCAAAUCAGAUUAUACA	1404	3029	UGUAUAAUUCUGAUUUUGCC	1580
3025	AGGAGUCUGCAUUUUGCACU	1405	3025	AGGAGUCUGCAUUUUGCACU	1405	3047	AGUGCAAUUGCAGACUCCU	1581
3043	UUUUUUUAGUGACUAAAGU	1406	3043	UUUUUUUAGUGACUAAAGU	1406	3065	ACUUUAGUCACUAAAAAA	1582
3061	UUGCUUAUUGAAACAUGU	1407	3061	UUGCUUAUUGAAACAUGU	1407	3083	ACAUGUUUUCAUUAAGCAA	1583
3079	UGCUGAAUUGUGGAAUUU	1408	3079	UGCUGAAUUGUGGAAUUU	1408	3101	AAAUCCACAACAUAUUCAGCA	1584
3097	UUGUGUAUAUAUUUACUUU	1409	3097	UUGUGUAUAUAUUUACUUU	1409	3119	AAAGUAAAUUAUAACACAA	1585
3115	UGUCCAGGAACUUUGGCAA	1410	3115	UGUCCAGGAACUUUGGCAA	1410	3137	UUGCACAAGUUCUUGGACA	1586
3133	AGGGAGAGCCAAAGGAAUA	1411	3133	AGGGAGAGCCAAAGGAAUA	1411	3155	UAUUUCCUUUGGCUUCUCCU	1587
3148	AAUAGGAUUGUUUGGCACCC	1412	3148	AAUAGGAUUGUUUGGCACCC	1412	3170	GGGUGGCCAAACAUAUCCUUAU	1588

The 3'-ends of the Upper sequence and the Lower sequence of the siRNA construct can include an overhang sequence, for example about 1, 2, 3, or 4 nucleotides in length, preferably 2 nucleotides in length, wherein the overhanging sequence of the lower sequence is optionally complementary to a portion of the target sequence. The upper sequence is also referred to as the sense strand, whereas the lower sequence is also referred to as the antisense strand. The upper and lower sequences in the Table can further comprise a chemical modification having Formulae I-VII or any combination thereof.



Table III: BCR-ABL and ERG Synthetic Modified siNA constructs

## BCR-ABL

Target Pos	Target	Seq ID	Aliases	Sequence	Seq ID
281	UGACCAUCAUAAGGAAGAGCC	1589	b2a2:283U21 siRNA sense	ACCAUCAUAAGGAAGAGTT	1601
284	CCAUCUAUAAGGAAGAGCCCUU	1590	b2a2:286U21 siRNA sense	AUCAUAAGGAAGAGCCCTT	1602
280	CUGACCAUCAUAAGGAAGAGCC	1591	b2a2:282U21 siRNA sense	GACCAUCAUAAGGAAGAATT	1603
288	CAUAAGGAAGAGAGCCCUUCAGC	1592	b2a2:290U21 siRNA sense	AUAAGGAAGAGCCCUUCATT	1604
281	UGACCAUCAUAAGGAAGAGCC	1589	b2a2:301L21 siRNA (283C) antisense	CUUCUCCUUAUUGAUGGUUTT	1605
284	CCAUCUAUAAGGAAGAGCCCUU	1590	b2a2:304L21 siRNA (286C) antisense	GGGCUUCUCCUUAUUGAUTT	1606
280	CUGACCAUCAUAAGGAAGAGCC	1591	b2a2:300L21 siRNA (282C) antisense	UUCUUCUUAUUGAUGGUCTT	1607
288	CAUAAGGAAGAGAGCCCUUCAGC	1592	b2a2:308L21 siRNA (290C) antisense	UGAAGGGCUUCUCCUUAUTT	1608
281	UGACCAUCAUAAGGAAGAGCC	1589	b2a2:283U21 siRNA stab4 sense	B AccAucAAuAAGGAAGAGTT B	1609
284	CCAUCUAUAAGGAAGAGCCCUU	1590	b2a2:286U21 siRNA stab4 sense	B AUCAAuAAGGAAGAGGccTT B	1610
280	CUGACCAUCAUAAGGAAGAGCC	1591	b2a2:282U21 siRNA stab4 sense	B GAccAucAAuAAGGAAGAGTT B	1611
288	CAUAAGGAAGAGAGCCCUUCAGC	1592	b2a2:290U21 siRNA stab4 sense	B AUAAGGAAGAGGccuucATT B	1612
281	UGACCAUCAUAAGGAAGAGCC	1589	b2a2:301L21 siRNA (283C) stab5 antisense	CUUCUCCUUAUUGAUGGUtsT	1613
284	CCAUCUAUAAGGAAGAGCCCUU	1590	b2a2:304L21 siRNA (286C) stab5 antisense	GGGCUUCUCCUUAUUGAUsT	1614
280	CUGACCAUCAUAAGGAAGAGCC	1591	b2a2:300L21 siRNA (282C) stab5 antisense	UUCUCCUUAUUGAUGGUtsT	1615
288	CAUAAGGAAGAGAGCCCUUCAGC	1592	b2a2:308L21 siRNA (290C) stab5 antisense	UGAAGGGCUUCUCCUUAUsT	1616
281	UGACCAUCAUAAGGAAGAGCC	1589	b2a2:283U21 siRNA stab7 sense	B AccAucAAuAAGGAAGAGTT B	1617
284	CCAUCUAUAAGGAAGAGCCCUU	1590	b2a2:286U21 siRNA stab7 sense	B AUCAAuAAGGAAGAGGccTT B	1618
280	CUGACCAUCAUAAGGAAGAGCC	1591	b2a2:282U21 siRNA stab7 sense	B GAccAucAAuAAGGAAGAGTT B	1619
288	CAUAAGGAAGAGAGCCCUUCAGC	1592	b2a2:290U21 siRNA stab7 sense	B AUAAGGAAGAGGccuucATT B	1620
281	UGACCAUCAUAAGGAAGAGCC	1589	b2a2:301L21 siRNA (283C) stab11 antisense	CUUCUCCUUAUUGAUGGUtsT	1621
284	CCAUCUAUAAGGAAGAGCCCUU	1590	b2a2:304L21 siRNA (286C) stab11 antisense	GGGCUUCUCCUUAUUGAUsT	1622
280	CUGACCAUCAUAAGGAAGAGCC	1591	b2a2:300L21 siRNA (282C) stab11 antisense	UUCUCCUUAUUGAUGGUtsT	1623

288	CAUAAGGAAGAAGCCCUUCAGC	1592	b2a2:308L21 siRNA (280C) stab11 antisense	uGAAGGGGcucucuccuuAuTsT	1624
354	UGGAUUUAAGCAGAGUUCAAAAG	1593	b3a2:356U21 siRNA sense	GAUUUAAGCAGAGUUCAAAATT	1625
363	GCAGAGUUCAAAAGCCCUUCAGC	1594	b3a2:365U21 siRNA sense	AGAGUUCAAAAGCCCUUCATT	1626
362	AGCAGAGUUCAAAAGCCCUUCAG	1595	b3a2:364U21 siRNA sense	CAGAGUUCAAAAGCCCUUCTT	1627
355	GGAUUUAAAGCAGAGUUCAAAAGC	1596	b3a2:357U21 siRNA sense	AUUUAAGCAGAGUUCAAAATT	1628
354	UGGAUUUAAGCAGAGUUCAAAAG	1593	b3a2:374L21 siRNA (356C) antisense	UUUGAACUCUCUGCUUAAAUCCTT	1629
363	GCAGAGUUCAAAAGCCCUUCAGC	1594	b3a2:383L21 siRNA (365C) antisense	UGAAGGGGCUUUUGAACUCUCUTT	1630
362	AGCAGAGUUCAAAAGCCCUUCAG	1595	b3a2:382L21 siRNA (364C) antisense	GAAGGGCUUUUGAACUCUCGTT	1631
355	GGAUUUAAAGCAGAGUUCAAAAGC	1596	b3a2:375L21 siRNA (357C) antisense	UUUUUGAACUCUCUGCUUAAAUCCTT	1632
354	UGGAUUUAAGCAGAGUUCAAAAG	1593	b3a2:356U21 siRNA stab4 sense	B GAUUUAAGCAGAGUUCAAAATT B	1633
363	GCAGAGUUCAAAAGCCCUUCAGC	1594	b3a2:365U21 siRNA stab4 sense	B AGAGUUCAAAAGCCCUUCATT B	1634
362	AGCAGAGUUCAAAAGCCCUUCAG	1595	b3a2:364U21 siRNA stab4 sense	B cAGAGUUCAAAAGCCCUUCTT B	1635
355	GGAUUUAAAGCAGAGUUCAAAAGC	1596	b3a2:357U21 siRNA stab4 sense	B AUUUUAAGCAGAGUUCAAAATT B	1636
354	UGGAUUUAAGCAGAGUUCAAAAG	1593	b3a2:374L21 siRNA (356C) stab5 antisense	uuuGAAAcucuGcuuAAAucTsT	1637
363	GCAGAGUUCAAAAGCCCUUCAGC	1594	b3a2:383L21 siRNA (365C) stab5 antisense	uGAAGGGGcuuuuGAAAcucuTsT	1638
362	AGCAGAGUUCAAAAGCCCUUCAG	1595	b3a2:382L21 siRNA (364C) stab5 antisense	GAAGGGGcuuuuGAAAcucuGTsT	1639
355	GGAUUUAAAGCAGAGUUCAAAAGC	1596	b3a2:375L21 siRNA (357C) stab5 antisense	uuuuGAAAcucuGcuuAAAucTsT	1640
354	UGGAUUUAAGCAGAGUUCAAAAG	1593	b3a2:356U21 siRNA stab7 sense	B GAUUUAAGCAGAGUUCAAAATT B	1641
363	GCAGAGUUCAAAAGCCCUUCAGC	1594	b3a2:365U21 siRNA stab7 sense	B AGAGUUCAAAAGCCCUUCATT B	1642
362	AGCAGAGUUCAAAAGCCCUUCAG	1595	b3a2:364U21 siRNA stab7 sense	B cAGAGUUCAAAAGCCCUUCTT B	1643
355	GGAUUUAAAGCAGAGUUCAAAAGC	1596	b3a2:357U21 siRNA stab7 sense	B AUUUUAAGCAGAGUUCAAAATT B	1644
354	UGGAUUUAAGCAGAGUUCAAAAG	1593	b3a2:374L21 siRNA (356C) stab11 antisense	uuuGAAAcucuGcuuAAAucTsT	1645
363	GCAGAGUUCAAAAGCCCUUCAGC	1594	b3a2:383L21 siRNA (365C) stab11 antisense	uGAAGGGGcuuuuGAAAcucuTsT	1646
362	AGCAGAGUUCAAAAGCCCUUCAG	1595	b3a2:382L21 siRNA (364C) stab11 antisense	GAAGGGGcuuuuGAAAcucuGTsT	1647
355	GGAUUUAAAGCAGAGUUCAAAAGC	1596	b3a2:375L21 siRNA (357C) stab11 antisense	uuuuGAAAcucuGcuuAAAucTsT	1648

## ERG

Target Pos	Target	SeqID	RP#	Aliases	Sequence	SeqID
242	AGGUGAAUGGCUCACGAAGAACUCU	1597	31045	ERG2:244U21 siRNA sense	GUGAAUGGCUCACGAAGAACUTT	1649
517	AAGGAACUGUGCAAGGAUGACCAA	1598	31046	ERG2:519U21 siRNA sense	GGAAUCUGUGCAAGGAUGACCTT	1650
759	GAAAGCUGCUCACCAACUCCUU	1599	31047	ERG2:761U21 siRNA sense	AAGCUGCUCACCAACUCCCTT	1651
767	CUCAACCAUCUCUCCUCCACAGUG	1600	31048	ERG2:769U21 siRNA sense	CAACCAUCUCCUCCACAGTT	1652
242	AGGUGAAUGGCUCACGAAGAACUCU	1597	31121	ERG2:262L21 siRNA (244C) antisense	AGUUCUUGAGCCAUUCACCTT	1653
517	AAGGAACUGUGCAAGGAUGACCAA	1598	31122	ERG2:537L21 siRNA (519C) antisense	GGUCAUCUUGCACAGUUCCTT	1654
759	GAAAGCUGCUCACCAACUCCUU	1599	31123	ERG2:779L21 siRNA (761C) antisense	GGAGAUGGUGAGCAGCUUTT	1655
767	CUCAACCAUCUCUCCUCCACAGUG	1600	31124	ERG2:787L21 siRNA (769C) antisense	CUGUGGAAGGAGAUUGGUUGTT	1656
242	AGGUGAAUGGCUCACGAAGAACUCU	1597	30761	ERG2:244U21 siRNA stab04 sense	B GUAAUGGUCUAAAGGAACUJT B	1657
517	AAGGAACUGUGCAAGGAUGACCAA	1598	30762	ERG2:519U21 siRNA stab04 sense	B GGAAUCUGUGCAAGGAUGACCTT B	1658
759	GAAAGCUGCUCACCAACUCCUU	1599	30763	ERG2:761U21 siRNA stab04 sense	B AAGCUGUCUAAACCAUCUCCCTT B	1659
767	CUCAACCAUCUCUCCUCCACAGUG	1600	30764	ERG2:769U21 siRNA stab04 sense	B CAACCAUCUCCUCCACAGTT B	1660
242	AGGUGAAUGGCUCACGAAGAACUCU	1597	30765	ERG2:262L21 siRNA (244C) stab05 antisense	AGUUCUUGAGCCAUUCACTsT	1661
517	AAGGAACUGUGCAAGGAUGACCAA	1598	30766	ERG2:537L21 siRNA (519C) stab05 antisense	GGUCAUUUUcAcAGUUCCTsT	1662
759	GAAAGCUGCUCACCAACUCCUU	1599	30767	ERG2:779L21 siRNA (761C) stab05 antisense	GGAGAUUGUUGAGCAGCUUsT	1663
767	CUCAACCAUCUCUCCUCCACAGUG	1600	30768	ERG2:787L21 siRNA (769C) stab05 antisense	cuGUUGGAAGGAGAUUGGUUGTsT	1664
242	AGGUGAAUGGCUCACGAAGAACUCU	1597		ERG2:244U21 siRNA stab07 sense	B GUAAUUGGUCUAAAGGAACUJT B	1665
517	AAGGAACUGUGCAAGGAUGACCAA	1598		ERG2:519U21 siRNA stab07 sense	B GGAAUCUGUGCAAGGAUGACCTT B	1666
759	GAAAGCUGCUCACCAACUCCUU	1599		ERG2:761U21 siRNA stab07 sense	B AAGCUGUCUAAACCAUCUCCCTT B	1667
767	CUCAACCAUCUCUCCUCCACAGUG	1600		ERG2:769U21 siRNA stab07 sense	B CAACCAUCUCCUCCACAGTT B	1668
242	AGGUGAAUGGCUCACGAAGAACUCU	1597		ERG2:262L21 siRNA (244C) stab11 antisense	AGUUCUUGAGCCAUUCACTsT	1669

517	AAGGAACUGUGCAAGAUGACCAA	1598	ERG2:537L21 siRNA (519C) stab11 antisense	GGUcAucuuGcAcAGuuccTst	1670
759	GAAAGCUGCUCAACCAUCUCCUU	1599	ERG2:779L21 siRNA (761C) stab11 antisense	GGAGAUgGguUGAGcAGcuuTst	1671
767	CUCAACCAUCUCCUCCACAGUG	1600	ERG2:787L21 siRNA (769C) stab11 antisense	cuGUgGAAGGAGAUgGguuGTst	1672

**B2A2**

Target Pos	Target	Seq ID	Aliases	Sequence	Seq ID
281	UGACCAUCAUAAGGAAGAGCC	1589	b2a2:283U21 siRNA	ACCAUCAUAAGGAAGAGTT	1601
284	CCAUCAUAAGGAAGAGCCUU	1590	b2a2:286U21 siRNA	AUCAUAAGGAAGAGCCCTT	1602
280	CUGACCAUCAUAAGGAAGAGC	1591	b2a2:282U21 siRNA	GACCAUCAUAAGGAAGATT	1603
288	CAUAAGGAAGAGCCCUUCAGC	1592	b2a2:290U21 siRNA	AUAAGGAAGAGCCCUUCATT	1604
281	UGACCAUCAUAAGGAAGAGCC	1589	b2a2:301L21 siRNA (283C)	CUUCUCCUUAUUGAUGGUTT	1605
284	CCAUCAUAAGGAAGAGCCUU	1590	b2a2:304L21 siRNA (286C)	GGGUUCCUCCUUAUUGAUTT	1606
280	CUGACCAUCAUAAGGAAGAGC	1591	b2a2:300L21 siRNA (282C)	UUCUCCUUAUUGAUGGUCTT	1607
288	CAUAAGGAAGAGCCCUUCAGC	1592	b2a2:308L21 siRNA (290C)	UGAAGGGCCUUCUCCUUAUTT	1608
281	UGACCAUCAUAAGGAAGAGCC	1589	b2a2:283U21 siRNA stab4	B AccAucAAuAAGGAAGAGTT B	1609
284	CCAUCAUAAGGAAGAGCCUU	1590	b2a2:286U21 siRNA stab4	B AucAAuAAGGAAGAGAGccTT B	1610
280	CUGACCAUCAUAAGGAAGAGC	1591	b2a2:282U21 siRNA stab4	B GAccAucAAuAAGGAAGATT B	1611
288	CAUAAGGAAGAGCCCUUCAGC	1592	b2a2:290U21 siRNA stab4	B AUAAGGAAGAGAGccuucATT B	1612
281	UGACCAUCAUAAGGAAGAGCC	1589	b2a2:301L21 siRNA (283C) stab5	cuucuuccuuAuUGAuGGuTsT	1613
284	CCAUCAUAAGGAAGAGCCUU	1590	b2a2:304L21 siRNA (286C) stab5	GGGcuucuuccuuAuUGAuTsT	1614
280	CUGACCAUCAUAAGGAAGAGC	1591	b2a2:300L21 siRNA (282C) stab5	uuuuuuuuuuAuUGAuGGuTsT	1615
288	CAUAAGGAAGAGCCCUUCAGC	1592	b2a2:308L21 siRNA (290C) stab5	uGAAAGGGGcuucuuccuuAuTsT	1616
281	UGACCAUCAUAAGGAAGAGCC	1589	b2a2:283U21 siRNA stab7	B AccAucAAuAAGGAAGAGATT B	1617
284	CCAUCAUAAGGAAGAGCCUU	1590	b2a2:286U21 siRNA stab7	B AucAAuAAGGAAGAGAGccTT B	1618
280	CUGACCAUCAUAAGGAAGAGC	1591	b2a2:282U21 siRNA stab7	B GAccAucAAuAAGGAAGATT B	1619
288	CAUAAGGAAGAGCCCUUCAGC	1592	b2a2:290U21 siRNA stab7	B AUAAGGAAGAGAGccuucATT B	1620
281	UGACCAUCAUAAGGAAGAGCC	1589	b2a2:301L21 siRNA (283C) stab11	cuucuuccuuAuUGAuGGuTsT	1621
284	CCAUCAUAAGGAAGAGCCUU	1590	b2a2:304L21 siRNA (286C) stab11	GGGcuucuuccuuAuUGAuTsT	1622
280	CUGACCAUCAUAAGGAAGAGC	1591	b2a2:300L21 siRNA (282C) stab11	uuuuuuuuuuAuUGAuGGuTsT	1623
288	CAUAAGGAAGAGCCCUUCAGC	1592	b2a2:308L21 siRNA (290C) stab11	uGAAAGGGGcuucuuccuuAuTsT	1624
354	UGAUUUUAGCAGAGUUCAAAAG	1593	b3a2:356U21 siRNA	GAUUUAGGCAGAGUUCAAAATT	1625
363	GCAGAGUUCAAAAGCCCUUCAGC	1594	b3a2:365U21 siRNA	AGAGUUCAAAAGCCCUUCATT	1626
362	AGCAGAGUUCAAAAGCCCUUCAG	1595	b3a2:364U21 siRNA	CAGAGUUCAAAAGCCCUUCCTT	1627

355	GGAUUUUAGCAGAGUUCAAAAGC	1596	b3a2:357U21 siRNA	AUUUAAAGCAGAGUUCAAAATT	1628
354	UGGAUUUAGCAGAGUUCAAAAG	1593	b3a2:374L21 siRNA (356C)	UUUGAACUCUGCUUAAAUCCTT	1629
363	GCAGAGUUCAAAAGCCCUUCAGC	1594	b3a2:383L21 siRNA (365C)	UGAAGGGCUUUUGAACUCUUTT	1630
362	AGCAGAGUUCAAAAGCCCUUCAG	1595	b3a2:382L21 siRNA (364C)	GAAGGGCUUUUGAACUCUGTT	1631
355	GGAUUUUAGCAGAGUUCAAAAGC	1596	b3a2:375L21 siRNA (357C)	UUUUGAACUCUGCUUAAAATT	1632
354	UGGAUUUAGCAGAGUUCAAAAG	1593	b3a2:356U21 siRNA stab4	B GAUUUAGCAGAGUUCAAAATT B	1633
363	GCAGAGUUCAAAAGCCCUUCAGC	1594	b3a2:365U21 siRNA stab4	B AGAGUUCAAAAGCCCUUCATT B	1634
362	AGCAGAGUUCAAAAGCCCUUCAG	1595	b3a2:364U21 siRNA stab4	B cAGAGUUCAAAAGCCCUUCATT B	1635
355	GGAUUUUAGCAGAGUUCAAAAGC	1596	b3a2:357U21 siRNA stab4	B AUUUUAGCAGAGUUCAAAATT B	1636
354	UGGAUUUAGCAGAGUUCAAAAG	1593	b3a2:374L21 siRNA (356C) stab5	UUUGAACUCUGCUUAAAATT	1637
363	GCAGAGUUCAAAAGCCCUUCAGC	1594	b3a2:383L21 siRNA (365C) stab5	UGAAGGGCUUUUGAACUCUUTT	1638
362	AGCAGAGUUCAAAAGCCCUUCAG	1595	b3a2:382L21 siRNA (364C) stab5	GAAAGGGCUUUUGAACUCUUTT	1639
355	GGAUUUUAGCAGAGUUCAAAAGC	1596	b3a2:375L21 siRNA (357C) stab5	UUUUUAGCUCUGCUUAAAATT	1640
354	UGGAUUUAGCAGAGUUCAAAAG	1593	b3a2:356U21 siRNA stab7	B GAUUUAGCAGAGUUCAAAATT B	1641
363	GCAGAGUUCAAAAGCCCUUCAGC	1594	b3a2:365U21 siRNA stab7	B AGAGUUCAAAAGCCCUUCATT B	1642
362	AGCAGAGUUCAAAAGCCCUUCAG	1595	b3a2:364U21 siRNA stab7	B cAGAGUUCAAAAGCCCUUCATT B	1643
355	GGAUUUUAGCAGAGUUCAAAAGC	1596	b3a2:357U21 siRNA stab7	B AUUUUAGCAGAGUUCAAAATT B	1644
354	UGGAUUUAGCAGAGUUCAAAAG	1593	b3a2:374L21 siRNA (356C) stab11	UUUGAACUCUGCUUAAAATT	1645
363	GCAGAGUUCAAAAGCCCUUCAGC	1594	b3a2:383L21 siRNA (365C) stab11	UGAAGGGCUUUUGAACUCUUTT	1646
362	AGCAGAGUUCAAAAGCCCUUCAG	1595	b3a2:382L21 siRNA (364C) stab11	GAAAGGGCUUUUGAACUCUUTT	1647
355	GGAUUUUAGCAGAGUUCAAAAGC	1596	b3a2:375L21 siRNA (357C) stab11	UUUUUAGCUCUGCUUAAAATT	1648

Uppercase = ribonucleotide  
 u,c = 2'-deoxy-2'-fluoro U, C  
 T = deoxy T  
 B = inverted deoxy abasic  
 s = phosphorothioate linkage  
 A = deoxy Adenosine  
 G = deoxy Guanosine

**Table IV**  
**Non-limiting examples of Stabilization Chemistries for chemically modified siNA constructs**

Chemistry	pyrimidine	Purine	cap	p=S	Strand
"Stab 1"	Ribo	Ribo	-	5 at 5'-end 1 at 3'-end	S/AS
"Stab 2"	Ribo	Ribo	-	All linkages	Usually AS
"Stab 3"	2'-fluoro	Ribo	-	4 at 5'-end 4 at 3'-end	Usually S
"Stab 4"	2'-fluoro	Ribo	5' and 3'-ends	-	Usually S
"Stab 5"	2'-fluoro	Ribo	-	1 at 3'-end	Usually AS
"Stab 6"	2'-O-Methyl	Ribo	5' and 3'-ends	-	Usually S
"Stab 7"	2'-fluoro	2'-deoxy	5' and 3'-ends	-	Usually S
"Stab 8"	2'-fluoro	2'-O-Methyl	-	1 at 3'-end	Usually AS
"Stab 9"	Ribo	Ribo	5' and 3'-ends	-	Usually S
"Stab 10"	Ribo	Ribo	-	1 at 3'-end	Usually AS
"Stab 11"	2'-fluoro	2'-deoxy	-	1 at 3'-end	Usually AS

CAP = any terminal cap, see for example Figure 10.

All Stab 1-11 chemistries can comprise 3'-terminal thymidine (TT) residues

All Stab 1-11 chemistries typically comprise 21 nucleotides, but can vary as described herein.

S = sense strand

AS = antisense strand

Table V

A. 2.5  $\mu$ mol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	6.5	163 $\mu$ L	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 $\mu$ L	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 $\mu$ L	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 $\mu$ L	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 $\mu$ L	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

B. 0.2  $\mu$ mol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	15	31 $\mu$ L	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 $\mu$ L	45 sec	233 min	465 sec
Acetic Anhydride	655	124 $\mu$ L	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 $\mu$ L	5 sec	5 sec	5 sec
TCA	700	732 $\mu$ L	10 sec	10 sec	10 sec
Iodine	20.6	244 $\mu$ L	15 sec	15 sec	15 sec
Beaucage	7.7	232 $\mu$ L	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2  $\mu$ mol Synthesis Cycle 96 well Instrument

Reagent	Equivalents:DNA/ 2'-O-methyl/Ribo	Amount: DNA/2'-O- methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O- methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 $\mu$ L	60 sec	180 sec	360sec
S-Ethyl Tetrazole	70/105/210	40/60/120 $\mu$ L	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 $\mu$ L	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 $\mu$ L	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 $\mu$ L	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 $\mu$ L	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 $\mu$ L	NA	NA	NA

- 5
- Wait time does not include contact time during delivery.
  - Tandem synthesis utilizes double coupling of linker molecule

CLAIMS

What we claim is:

1. A double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a BCR-ABL gene, wherein said siNA molecule is about 21 nucleotides long.  
5
2. The siNA molecule of claim 1, wherein said siNA molecule comprises no ribonucleotides.
3. The siNA molecule of claim 1, wherein said siNA molecule comprises ribonucleotides.
- 10 4. The siNA molecule of claim 1, wherein one of the strands of said double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of a BCR-ABL gene, and wherein the second strand of said double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of said BCR-ABL gene.  
15
5. The siNA molecule of claim 4, wherein each said strand of the siNA molecule comprises about 19 to about 23 nucleotides, and wherein each said strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.
- 20 6. The siNA molecule of claim 1, wherein said siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of a BCR-ABL gene, and wherein said siNA further comprises a sense region, wherein said sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of said BCR-ABL gene.  
25
7. The siNA molecule of claim 6, wherein said antisense region and said sense region each comprise about 19 to about 23 nucleotides, and wherein said antisense region comprises at least about 19 nucleotides that are complementary to nucleotides of the sense region.

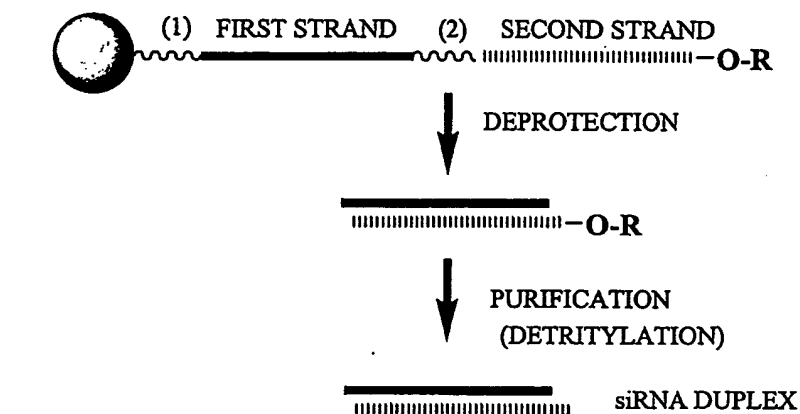


8. The siNA molecule of claim 1, wherein said siNA molecule comprises a sense region and an antisense region and wherein said antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of RNA encoded by a BCR-ABL gene and said sense region comprises a nucleotide sequence that is complementary to said antisense region.
9. The siNA molecule of claim 6, wherein said siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of said siNA molecule.
10. The siNA molecule of claim claim 6, wherein said sense region is connected to the antisense region via a linker molecule.
11. The siNA molecule of claim 10, wherein said linker molecule is a polynucleotide linker.
12. The siNA molecule of claim 10, wherein said linker molecule is a non-nucleotide linker.
13. The siNA molecule of claim 6, wherein pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides.
14. The siNA molecule of claim 6, wherein purine nucleotides in the sense region are 2'-deoxy purine nucleotides.
15. The siNA molecule of claim 6, wherein the pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides.
16. The siNA molecule of claim 9, wherein the fragment comprising said sense region includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment comprising said sense region.
17. The siNA molecule of claim 16, wherein said terminal cap moiety is an inverted deoxy abasic moiety.
18. The siNA molecule of claim 6, wherein the pyrimidine nucleotides of said antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides

19. The siNA molecule of claim 6, wherein the the purine nucleotides of said antisense region are 2'-O-methyl purine nucleotides.
20. The siNA molecule of claim 6, wherein the purine nucleotides present in said antisense region comprise 2'-deoxy- purine nucleotides.
- 5 21. The siNA molecule of claim 18, wherein said antisense region comprises a phosphorothioate internucleotide linkage at the 3' end of said antisense region.
22. The siNA molecule of claim 6, wherein said antisense region comprises a glyceryl modification at the 3' end of said antisense region.
- 10 23. The siNA molecule of claim 9, wherein each of the two fragments of said siNA molecule comprise 21 nucleotides.
24. The siNA molecule of claim 23, wherein about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule and wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule.
- 15 25. The siNA molecule of claim 24, wherein each of the two 3' terminal nucleotides of each fragment of the siNA molecule are 2'-deoxy-pyrimidines.
26. The siNA molecule of claim 25, wherein said 2'-deoxy-pyrimidine is 2'-deoxy-thymidine.
- 20 27. The siNA molecule of claim 23, wherein all 21 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule.
28. The siNA molecule of claim 23, wherein about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by a BCR-ABL gene.
- 25 29. The siNA molecule of claim 23, wherein 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by a BCR-ABL gene.

30. The siNA molecule of claim 9, wherein the 5'-end of the fragment comprising said antisense region optionally includes a phosphate group.
31. The siNA molecule of claim 1, wherein said BCR-ABL gene encodes sequence comprising Genbank Accession number NM\_004327 (BCR).
- 5 32. The siNA molecule of claim 1, wherein said BCR-ABL gene encodes sequence comprising Genbank Accession number NM\_005157 (ABL).
33. The siNA molecule of claim 1, wherein said BCR-ABL gene encodes sequence comprising Genbank Accession number HSA131467 (b2a2).
- 10 34. A double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a BCR-ABL gene, wherein said siNA molecule comprises no ribonucleotides and wherein each strand of said double-stranded siNA molecule is about 21 nucleotides long.
35. The siNA molecule of claim 34, wherein said BCR-ABL gene encodes sequence comprising Genbank Accession number NM\_004327 (BCR).
- 15 36. The siNA molecule of claim 34, wherein said BCR-ABL gene encodes sequence comprising Genbank Accession number NM\_005157 (ABL).
37. The siNA molecule of claim 34, wherein said BCR-ABL gene encodes sequence comprising Genbank Accession number HSA131467 (b2a2).
- 20 38. A double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a BCR-ABL gene, wherein said siNA molecule does not require the presence of a ribonucleotide within the siNA molecule for said inhibition of expression of the BCR-ABL gene and wherein each strand of said double-stranded siNA molecule is about 21 nucleotides long.
- 25 39. The siNA molecule of claim 38, wherein said BCR-ABL gene encodes sequence comprising Genbank Accession number NM\_004327 (BCR).
40. The siNA molecule of claim 38, wherein said BCR-ABL gene encodes sequence comprising Genbank Accession number NM\_005157 (ABL).

41. The siNA molecule of claim 38, wherein said BCR-ABL gene encodes sequence comprising Genbank Accession number HSA131467 (b2a2).
42. A pharmaceutical composition comprising the siNA molecule of claim 1 in an acceptable carrier or diluent.
- 5 43. Medicament comprising the siNA molecule of claim 1.
44. Active ingredient comprising the siNA molecule of claim 1.
45. Use of a double-stranded short interfering nucleic acid (siNA) molecule to down-regulate expression of a BCR-ABL gene, wherein said siNA molecule comprises one or more chemical modifications and each strand of said double-stranded siNA is  
10 about 21 nucleotides long.

**Figure 1**

= SOLID SUPPORT

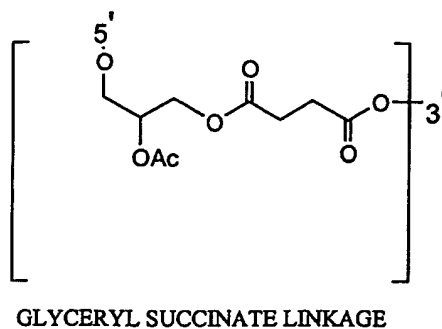
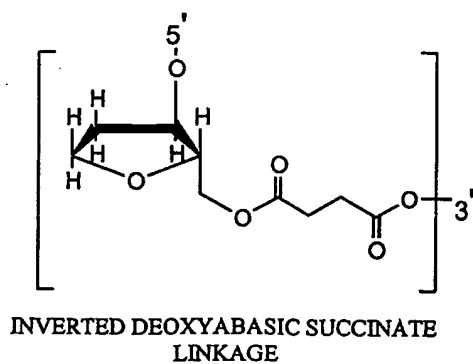
**R** = TERMINAL PROTECTING GROUP

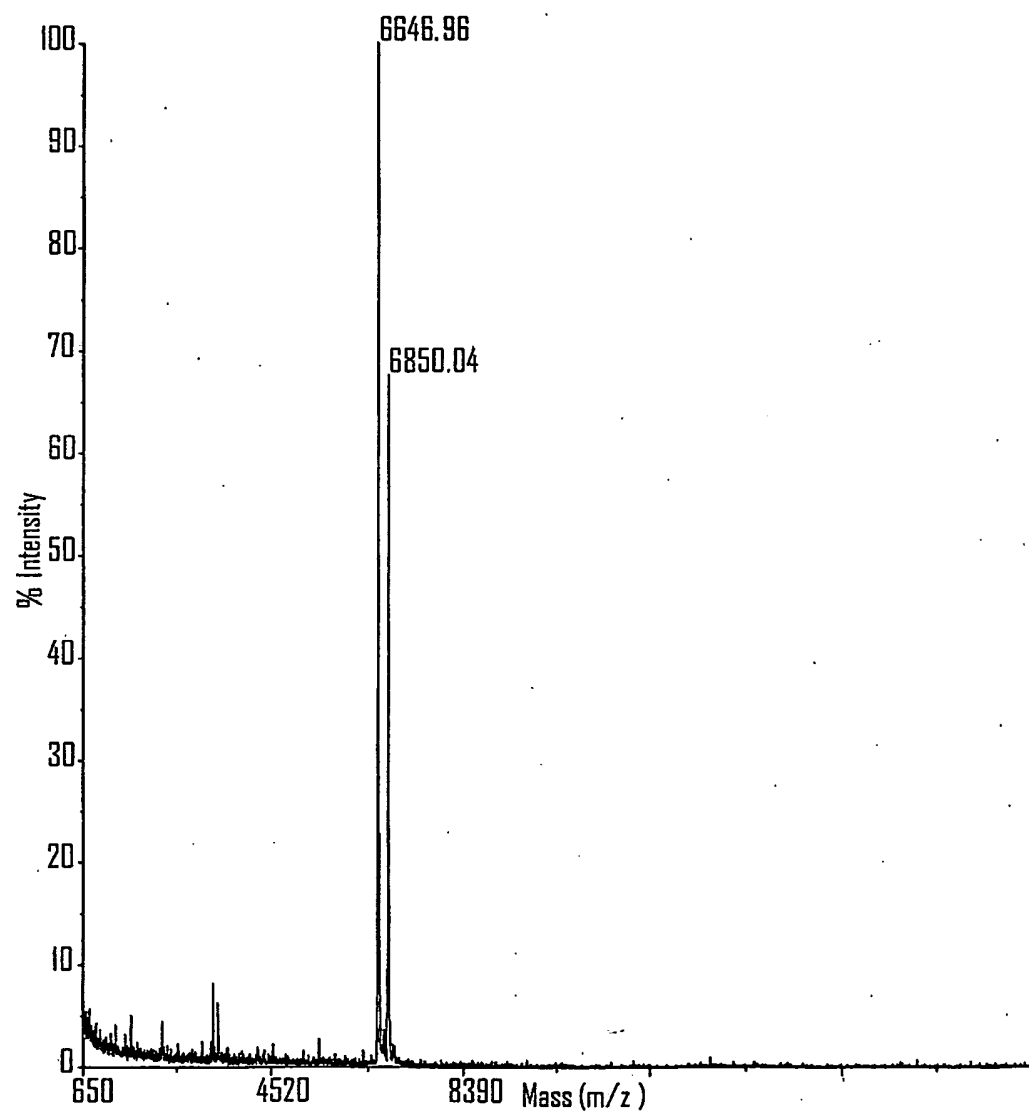
FOR EXAMPLE:

DIMETHOXYTRITYL (DMT)

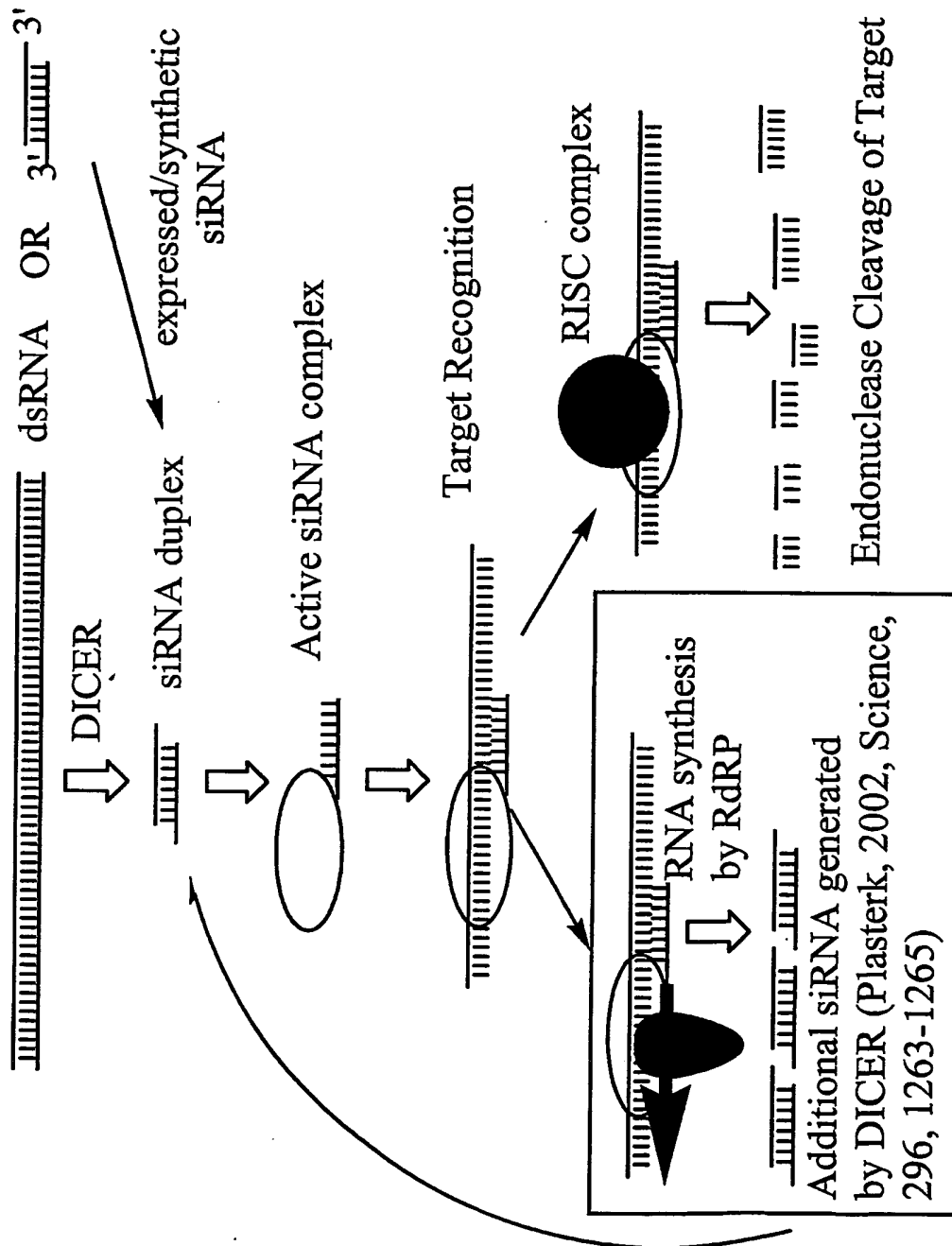
(1) = CLEAVABLE LINKER  
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR  
INVERTED DEOXYABASIC SUCCINATE)

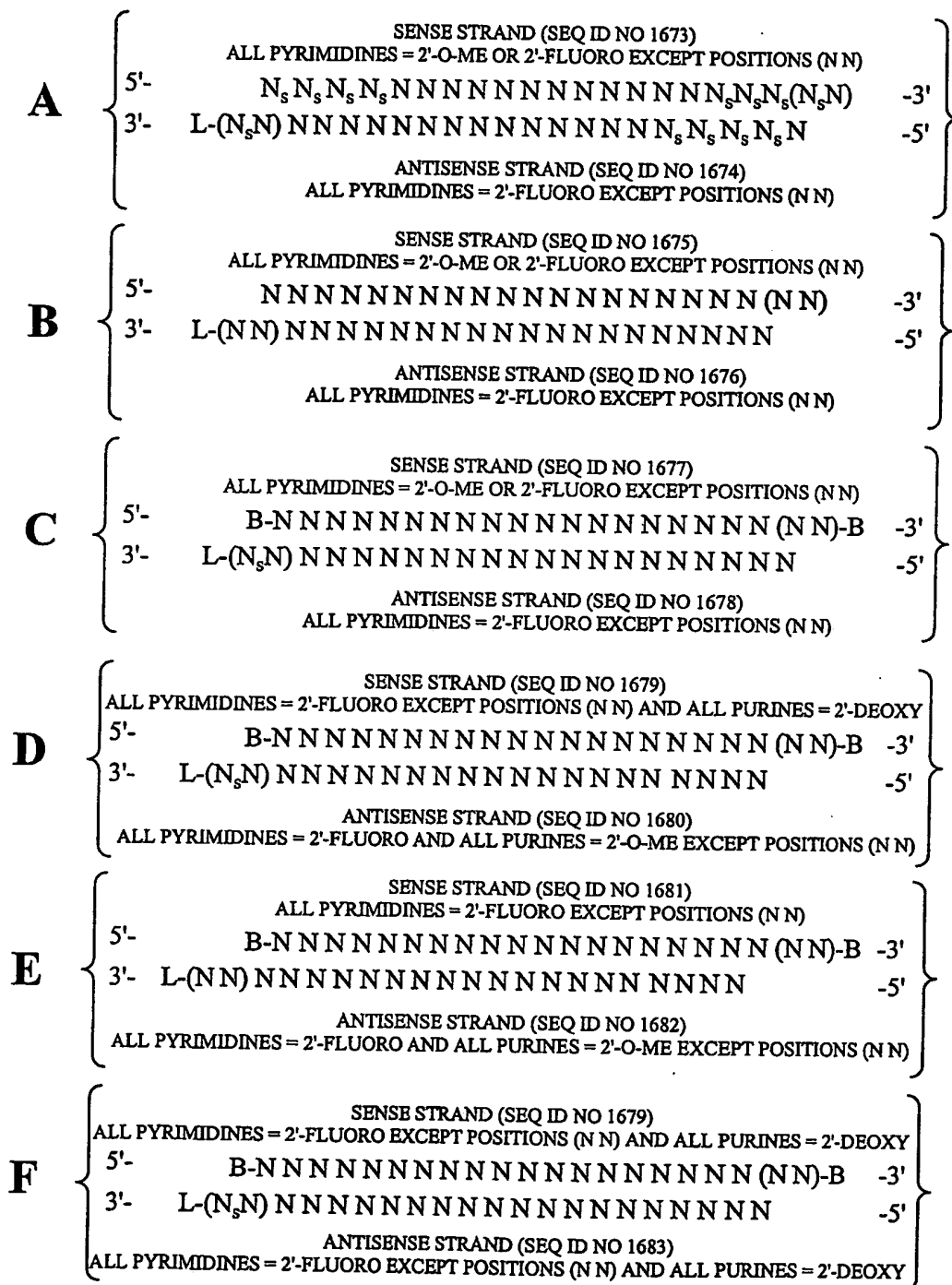
(2) = CLEAVABLE LINKER  
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR  
INVERTED DEOXYABASIC SUCCINATE)



*Figure 2*

**Figure 3**



**Figure 4**

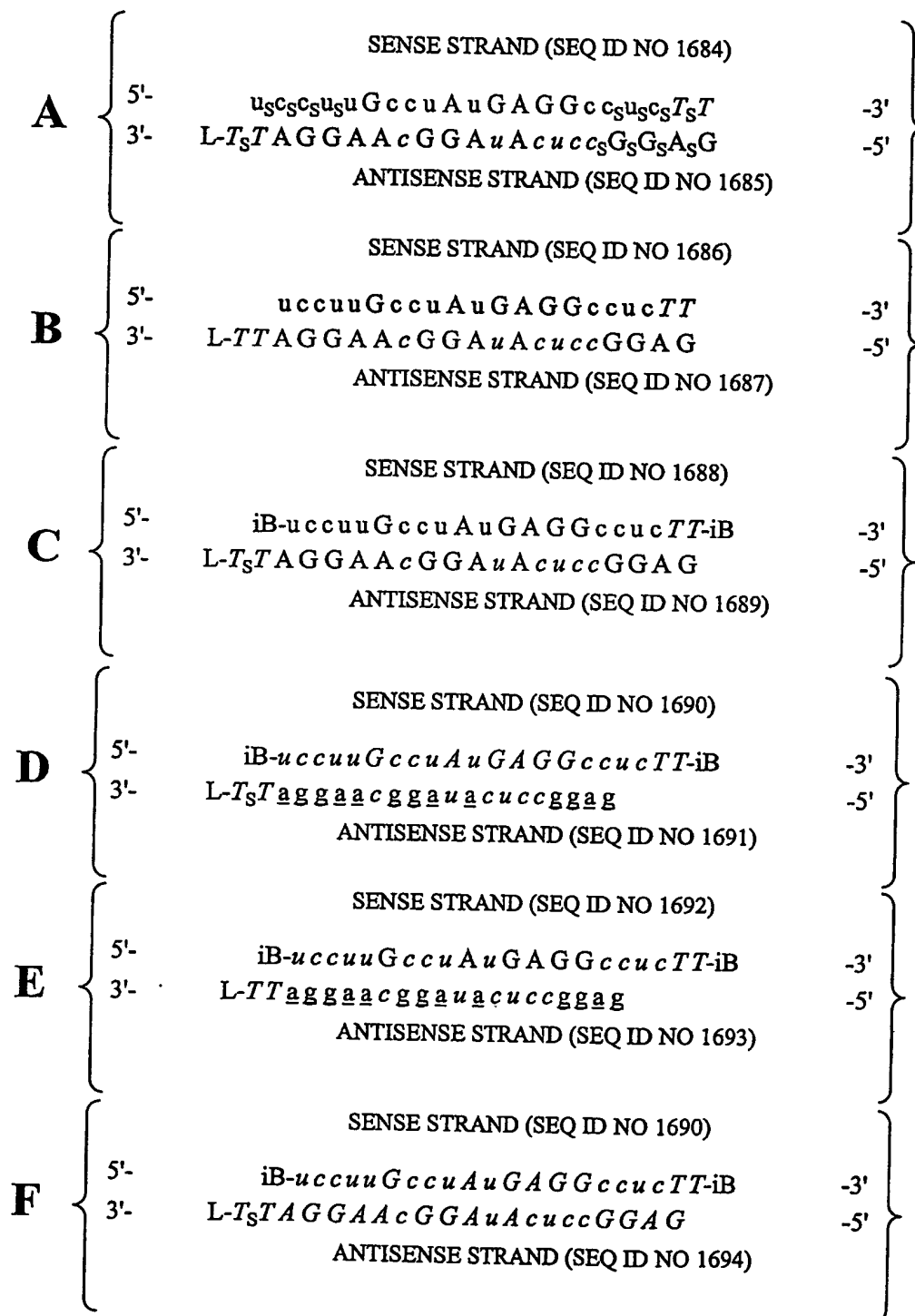
POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES

B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT

L = GLYCERYL MOIETY THAT IS OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE



**Figure 5**

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro

*italic lower case* = 2'-deoxy-2'-fluorounderline = 2'-O-methyl*ITALIC UPPER CASE* = DEOXY

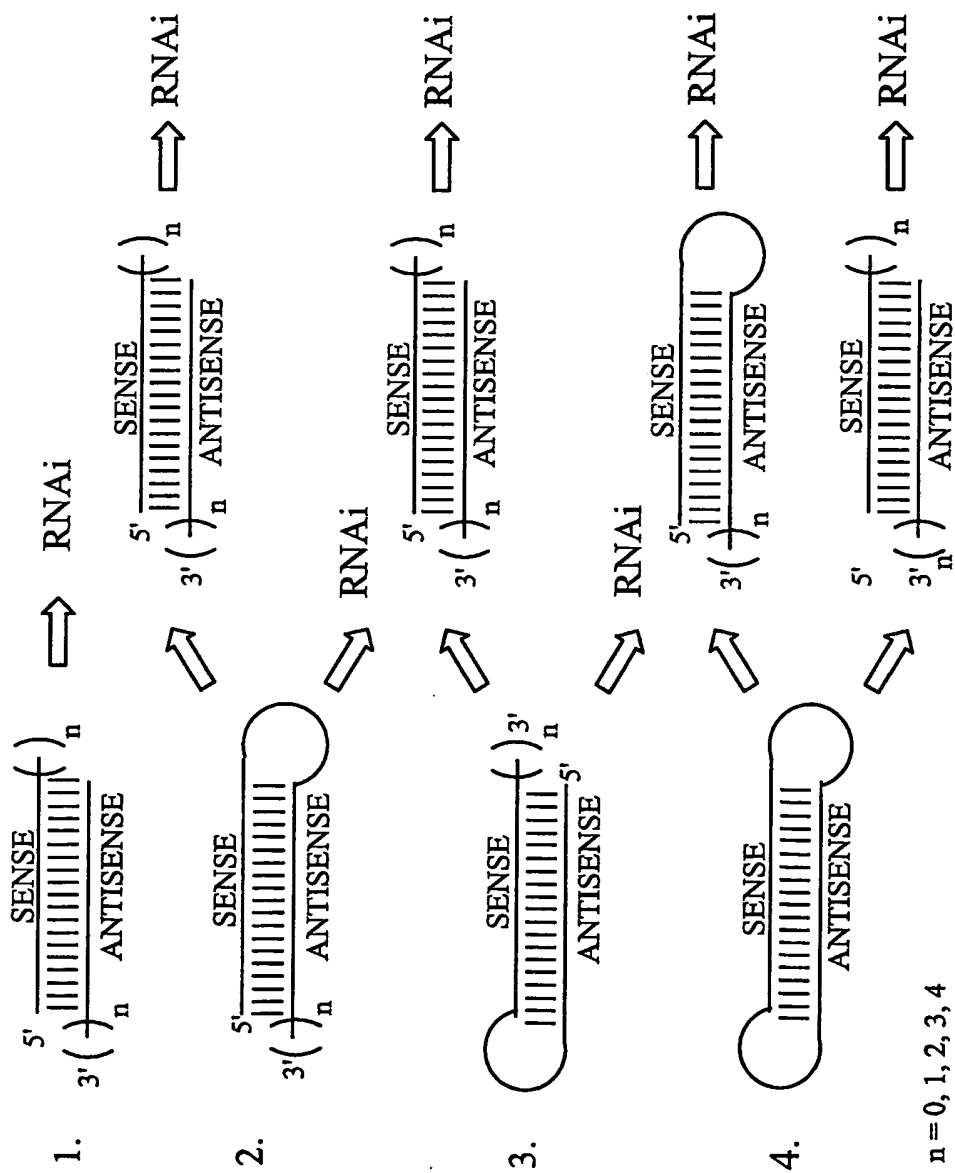
B = INVERTED DEOXYABASIC

L = GLYCERYL MOIETY OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR

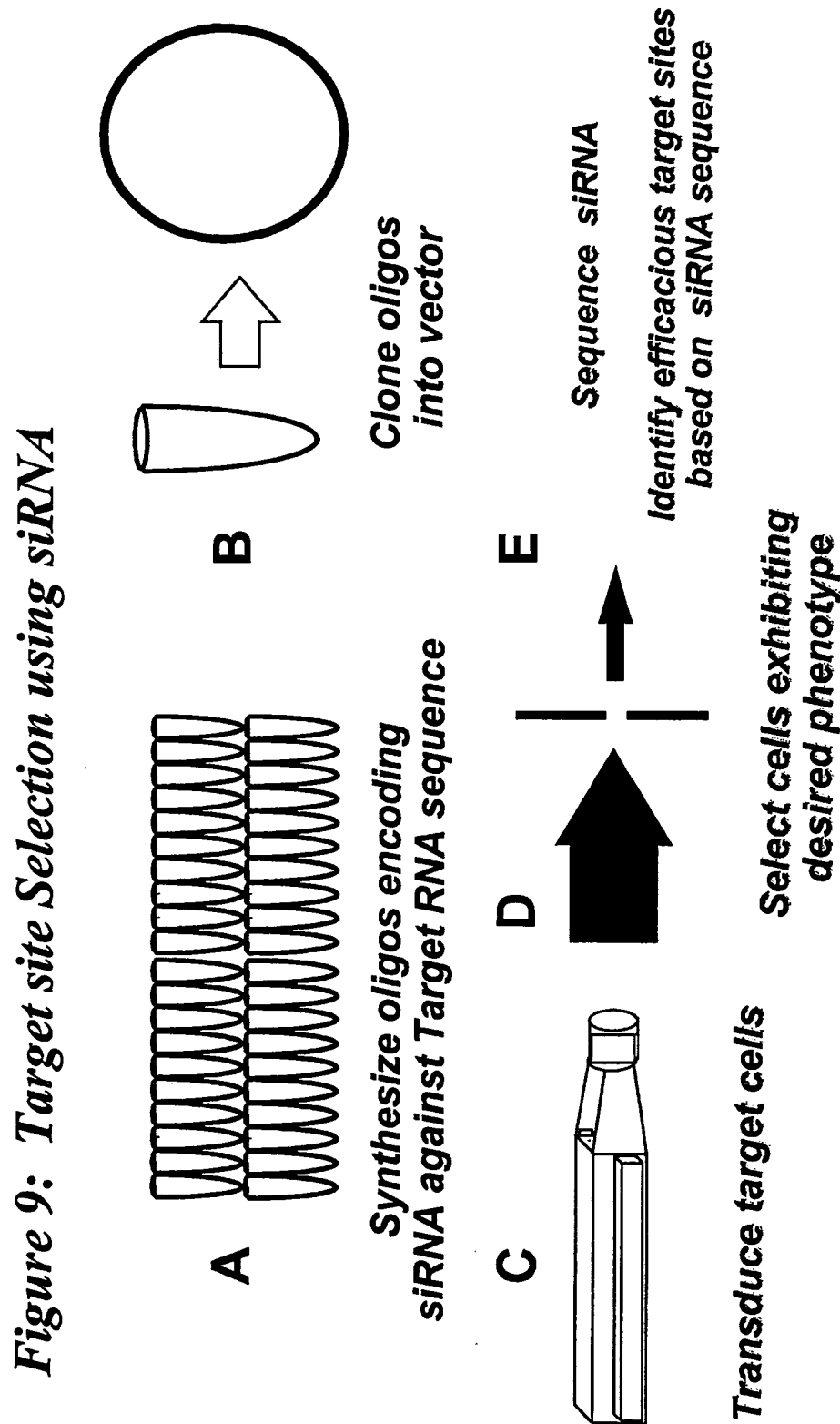
PHOSPHORODITHIOATE

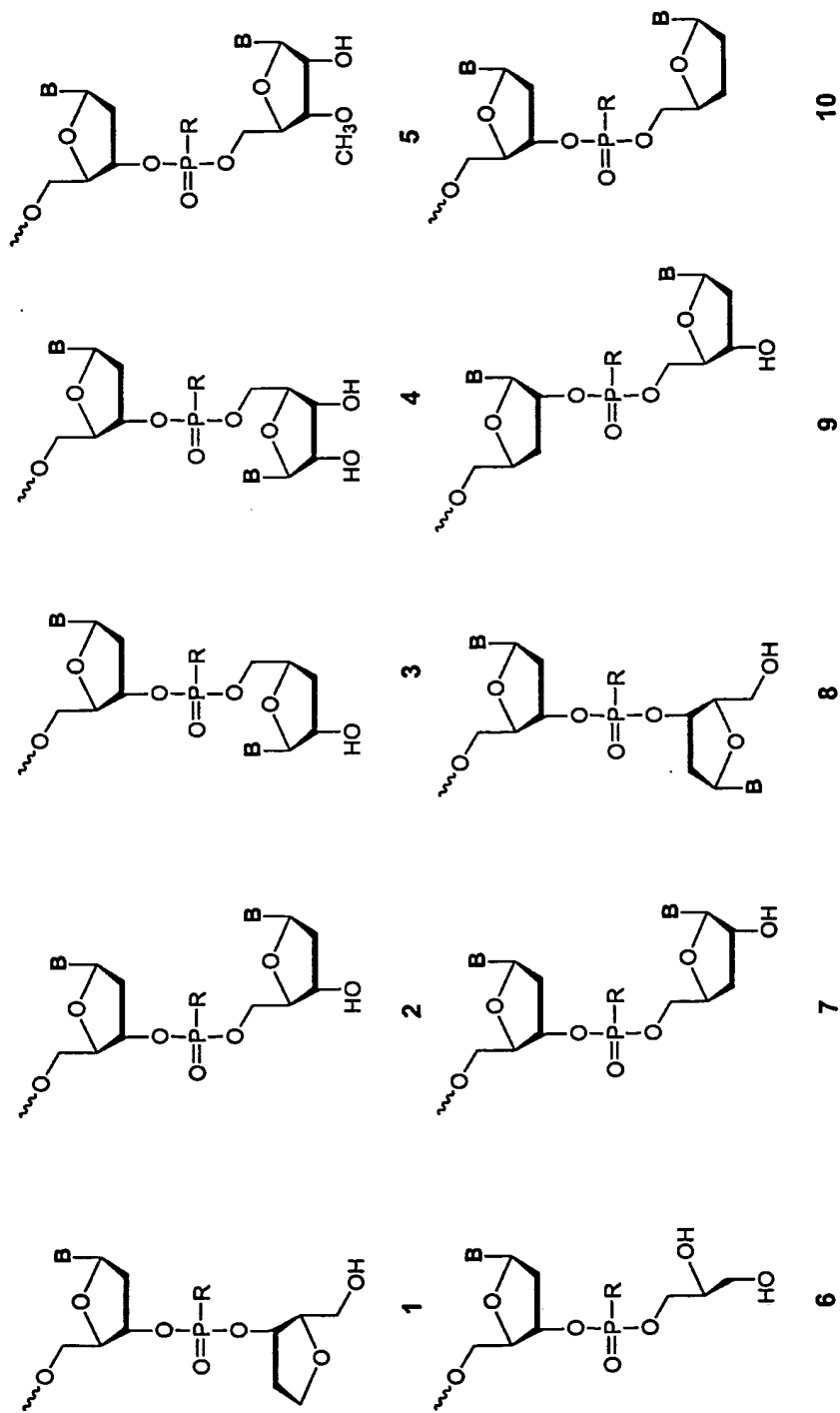
**Figure 6**







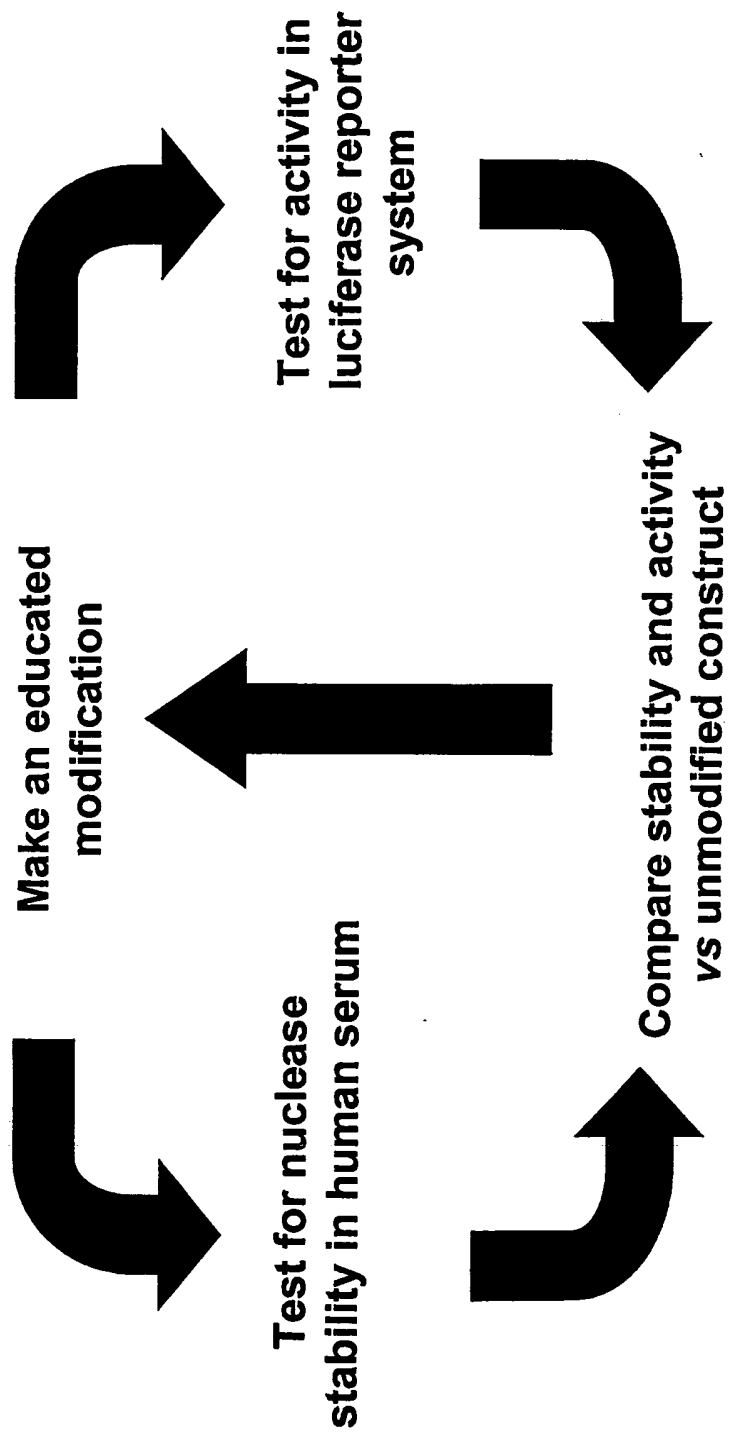


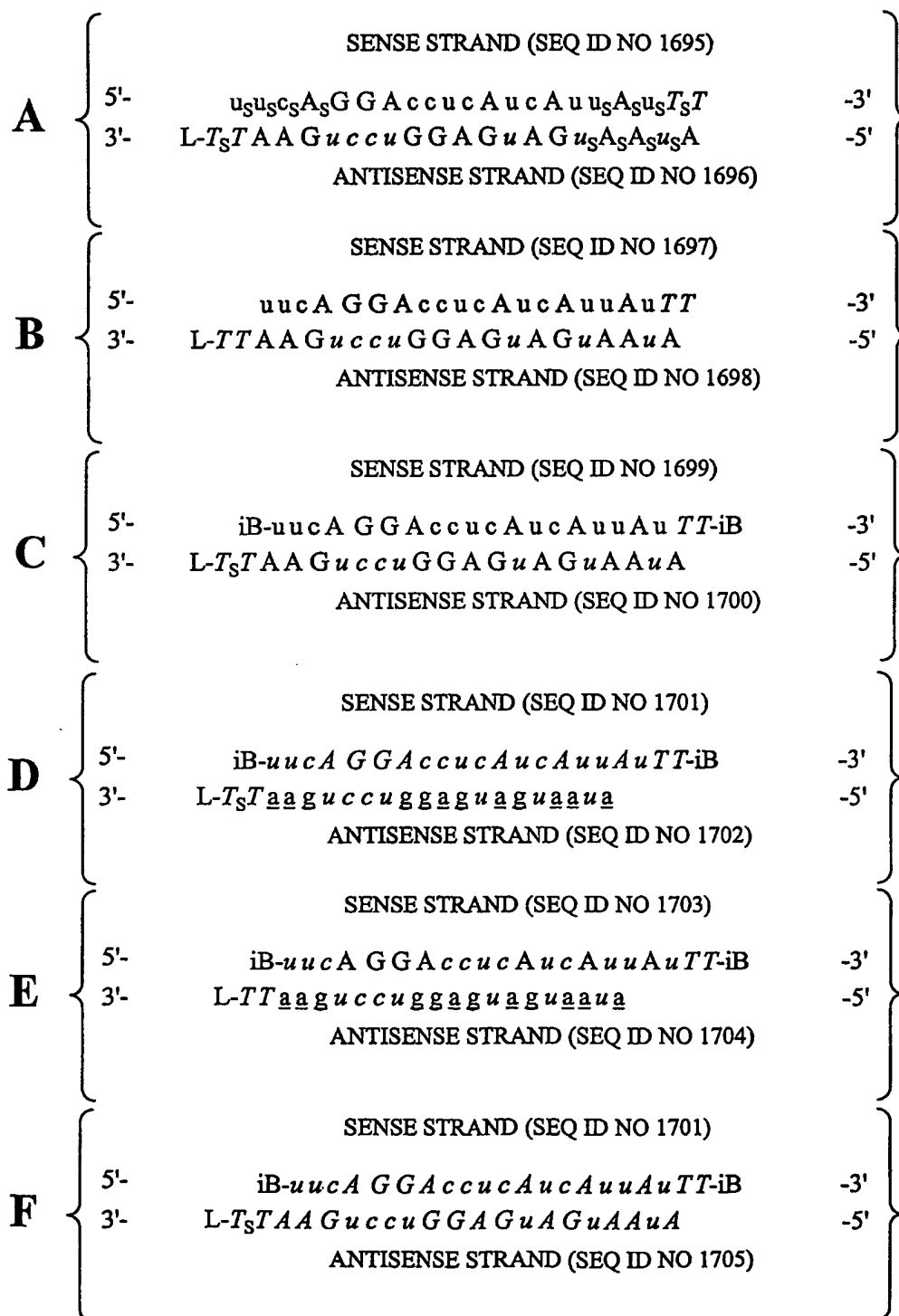
*Figure 10*

R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl

B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

*Figure 11: Modification Strategy*



**Figure 12**

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro

*italic lower case* = 2'-deoxy-2'-fluorounderline = 2'-O-methyl*ITALIC UPPER CASE* = DEOXY

B = INVERTED DEOXYABASIC

L = GLYCERYL MOIETY OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR

PHOSPHORODITHIOATE



**Figure 13: HeLa 24h ERG2 mRNA Expression**

